STUDIES ON *Solanum mauritianum* Scopoli BACTERIAL ENDOPHYTES AND THE EFFECTS OF THEIR CRUDE SECONDARY METABOLITES ON COMMON PATHOGENIC BACTERIA AND RESISTANT HUMAN CANCER CELLS.

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DECLARATION

I, Nkemdinma Chinezurum Uche-Okereafor hereby declare that the composition of this dissertation and the work herein described was carried out entirely by myself unless otherwise cited or acknowledged. It has not been submitted for degree purposes at any other University or institution. Every other source(s) used have been duly cited in text and acknowledged by complete references.

Nkemdinma Chinezurum Uche-Okereafor

DEDICATION

This work is dedicated to everyone who at one point or another offered a word of encouragement to me on this journey. Thank you.

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RESEARCH OUTPUTS

Journal Publications

1. **Nkemdinma Uche-Okereafor**, Tendani Sebola, Kudzanai Tapfuma, Lukhanyo Mekuto, Ezekiel Green and Vuyo Mavumengwana (2019). Antibacterial Activities of Crude Secondary Metabolite Extracts from *Pantoea* Species Obtained from the Stem of *Solanum mauritianum* and Their Effects on Two Cancer Cell Lines. *International Journal of Environmental Research and Public Health*, 2019, 16, 602. (Published).

2. **Nkemdinma Uche-Okereafor**, Tendani Sebola, Lukhanyo Mekuto, Maya Makatini, Ezekiel Green and Vuyo Mavumengwana (2020). Untargeted Secondary metabolite profiling, antibacterial and anticancer screening of crude secondary metabolite extracts of *Solanum mauritianum* Scop. aerial parts and their bacterial endophytes. *Transactions of the Royal Society of South Africa* (In review).

3. **Nkemdinma Uche-Okereafor**, Tendani Sebola, Lukhanyo Mekuto, Maya Makatini, Ezekiel Green and Vuyo Mavumengwana (2020). Antibacterial and Anticancer Activity of *Solanum mauritianum* Scop. ripe fruit coat and identification of bioactive secondary metabolites using LC-QTOF-MS/MS. *Advances in Pharmacological and Pharmaceutical Sciences Journal* (In review).

4. **Nkemdinma Uche-Okereafor**, Tendani Sebola, Lukhanyo Mekuto, Maya Makatini, Ezekiel Green and Vuyo Mavumengwana (2020). Identification of bioactive secondary metabolites present in the crude extracts of endophytic bacterial species isolated from the aerial parts of *Solanum mauritianum* Scop. *Journal of Chemical Technology and Biotechnology* (Prepared for publication).

Conferences

1. 69th Canadian Society of Microbiology (CSM) conference **"Effects of crude secondary metabolites extracted from *Solanum mauritianum* and its bacterial endophytes on pathogenic bacteria and resistant human cancer cells."** Sherbrooke, Quebec, Canada; June 10 – 13, 2019.

2. 2019 Bio Africa Convention, **"Antibacterial and anticancer activities of the ripe fruit cover of *Solanum mauritianum* on selected pathogenic bacteria and resistant human cancer cells and its LC-QTOF-MS screening."** Durban, South Africa; August 25 – 28, 2019.

3. The 3rd Women in STEM (WIS) conference **"Antibacterial and anticancer activities of crude extracts from endophytic *Pantoea* species obtained from the stem of *Solanum mauritianum*."** East London, South Africa; February 17 – 20, 2020.

GENERAL ABSTRACT

Plants are known to contain bioactive chemicals that can be used to improve health. In recent times, it has been reported that not only plants but plant symbionts (endophytes) can also produce bioactive chemicals. Endophytes are microorganisms that are perceived as non-pathogenic symbionts found inside plants and they cause no symptoms of disease on the host plant. Endophytes can take up the plant's DNA and are able to produce secondary metabolites that can sometimes be similar to the host plants counterparts. Because of the role that the endophytes' secondary metabolites have on the physiology of their host plant, they therefore have the potential for applications in the agriculture, biotechnology and pharmaceutical industries. Our interest in this study is on the medicinal plant *Solanum mauritianum* which is an invasive weedy plant species from the family Solanaceae that has been reported to be useful in South African traditional medicine for the treatment of menorrhagia, dysentery, diarrhoea, infertility, STIs: Gonorrhea, Chlamydia, Syphilis. The main aim of this study was to determine the bacterial endophytes diversity of *S. mauritianum* and the antibacterial activity of both the plant and endophytes' secondary metabolite crude extracts on selected pathogenic bacteria and human cancer cells, as well as identify and compare the type of bioactive secondary metabolites in the crude extracts.

Fresh, healthy aerial parts (leaves, ripe and unripe fruits and stems) of *S. mauritianum* were collected, washed, surface sterilized, macerated in PBS, inoculated on nutrient agar plates, and incubated for 5 days at 30˚C. Amplification and sequencing of the 16S rRNA gene was applied to identify the isolated bacterial endophytes. Crude secondary metabolite production was undertaken by growing the isolates in nutrient broth 7-14 days subsequent to the addition the Amberlite® XAD7HP 20-60 mesh resin for the adsorption of the secondary metabolites. These metabolites were extracted using ethyl acetate. The different collected plant parts were ground to fine powder and extracted using Choloform:Methanol (1:1, v/v). Thereafter, the crude metabolites from each of the isolated endophytes and plant parts were concentrated and tested against 11 pathogenic bacteria for antibacterial activity using the minimum inhibitory concentration (MIC) method, tested against two human cancer cell lines: Glioblastoma and Lung carcinoma cell lines using the MTS assay and finally the extracts were screened for the presence of secondary metabolites using LC-QTOF-MS/MS.

In this study, a total of 7 bacterial endophytes were isolated and identified. Since the control plates did not reveal any bacterial growth, it was concluded that the isolates reported are endophytic to *S. mauritianum*. The bacterial endophytes identified included species of the *Pantoea, Xanthomonas*, *Arthrobacter* and *Bacillus* genera. The antibacterial assay showed that the extracts were very active against the pathogenic microbes used in this study with MIC values ranging from 0.031 – 0.500 mg/mL. The extracts did not show any noteworthy activity against the cancer cells with the most activity being a reduction in cell viability by 37.9417 % at 100 µg/mL observed for *S. mauritianum* ripe fruit coat. The cell viability of the plant and endophytic samples at concentrations 100 - 3.13 µg/mL ranged from 62.0582 – 308.9493 % for glioblastoma cells and from 67-1244 – 135.4244 % for lung carcinoma cells. Statistical analysis of the concentration based activity of the crude bacterial and plant extracts showed that $P \geq 0.05$ indicating that the there is no significance in their cytotoxic effects on the cancer cells. The untargeted metabolite screening of the crude extracts identified 46 compounds in the plant parts alone, 38 compounds in the endophytic bacterial species alone and recorded 11 compounds occurring in both the plants parts and endophytes. Of the 38 compounds identified in the endophytes, the phytochemical classes most prevalent include: alkaloids (36.7 %), terpenoids (22.4 %) and flavonoids (16.3 %) while in the plant, the most prevalent phytochemical classes out of the 46 identified compounds are: alkaloids (43.8 %) and terpenoids (19.2 %). The compounds identified in both the bacterial endophytes and their host plant parts include: anatabine, swainsonine, neoquassin, genipin, droserone, loganin, hetisine, columbin, tuliposide A, montanol and cannabielsoin most of which have shown potential in pharmacological uses as like anti-microbial, anti-cancer, anti-inflammatory, etc. compounds.

The results of this study shows that both plants and their resident endophytes can be useful sources of drug discovery research and drug development seeing as the crude extracts of both the isolated bacterial endophytes and *S. mauritianum* plant parts had significant activity against common pathogenic bacteria and significant phytochemical compounds were also identified in the crude extracts.

Keywords: Antibacterial, anticancer, endophytes, glioblastoma, LC-QTOF-MS/MS, lung carcinoma, secondary metabolites, *Solanum mauritianum*.

OUTLINE OF DISSERTATION

The following chapters are presented in this thesis:

Chapter 1: General introduction

In this chapter, a general information on the background of this study is discussed while highlighting the justification, hypothesis aims and objectives of this study.

Chapter Two: Literature review

This chapter discusses the importance of medicinal plants and endophytes. It gives insight on the importance of secondary metabolites from plants and endophytes, the different secondary metabolites isolated/extracted from endophytes, the *Solanum mauritianum* plant.

Chapter 3: Isolation and characterization of Bacterial endophytes from *Solanum mauritianum* Scop.

This chapter highlights the experimental and analytical techniques used in this study for the isolation and identification of the bacterial endophytes present in *S. mauritianum*.

Chapter 4: Antibacterial activities and cytotoxicity of secondary metabolites from *Solanum mauritianum* Scop. and its bacterial endophytes on selected bacterial pathogens, Glioblastoma and Lung carcinoma cancer cell lines.

This chapter explains experimental and analytical techniques used in the antibacterial and anticancer activity screening of crude secondary metabolites extracted from *S. mauritianum* and its bacterial endophytes. The results of the screening are also discussed in this chapter.

Chapter 5: Untargeted secondary metabolite profiling of crude extracts from isolated bacterial endophytes and *Solanum mauritianum* plant parts using liquid chromatography coupled to quadrupole time-of-flight with tandem mass spectrometry (LC-QTOF-MS/MS).

In this chapter, the crude secondary metabolites of *S. mauritianum* plant and its bacterial endophytes are screened for secondary metabolites. The experimental and analytical techniques used for this screening are explained in this chapter.

Chapter 6: General discussions, conclusions, and recommendations for future work

This chapter covers the general discussions and conclusion from this study and the recommendations for future work.

Appendices

This contains other data generated in this study which includes: gene sequences of isolated endophytic bacteria, MTS assay data showing the percentage cell viability and stand deviation of the analysed samples and the different chromatograms of identified secondary metabolites in both endophytes and plants.

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
AMF	Arbuscular Mycorrhizal Fungi
CSCs	Cancer stem cells
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
GB	Glioblastoma
ha	Hectare
HPLC	High-performance liquid chromatography
h	Hours
HCN	Hydrocyanic acid
LC-MS	Liquid chromatography–mass spectrometry
LC-QTOF	Liquid chromatography quadrupole time of flight
LC-QTOF-MS/MS	Liquid chromatography coupled to quadrupole time-of-flight mass spectrometry
MIC	Minimum Inhibitory Concentrations
mins	minutes
MH	Mueller Hinton
NCI	National Cancer Institute
NCBI	National Center for Biotechnology Information
NJ	Neighbor Joining
NA	Nutrient Agar
PBS	Phosphate buffered saline
PGP	Plant growth promotion
PGPR	Plant growth-promoting rhizobacteria
PCR	Polymerase chain reaction
PVDF	Polyvinylidene fluoride
RTCA	Real-time cell analyser
rcf	Relative Centrifugal Force

rRNA	Ribosomal ribonucleic acid
NaHClO	Sodium Hypochlorite
SP1	Specificity protein 1
TM	Traditional medicine
UV	Ultra violet
WHO	World Health Organization

LIST OF SYMBOLS AND UNITS

Å	angstrom
cells/mL	cells per milliliter
cm	centimeters
˚C	degrees Celsius
g	gram
g/L	gram/Litre
>	Greater than
≥	greater than or equal to
kV	kilovolt
≤	less than or equal to
L/min	Liter per minute
m/z	Mass to charge ratio
m	meters
µg/mL	microgram per milliliter
µL	microliter
µM	micrometer
mg	milligram
mg/kg	Milligram per kilogram
mg/mL	milligram per milliliter
mL	milliliter
mL/min	milliliter per minute
mm	millimeters
nm	Nanometer
N_2	Nitrogen
%	percent
±	Plus, or minus
K+	Potassium ion
Na+	Sodium ions
V	volt

v/v	volume by volume
H_2O	water
w/v	weight by volume

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 BACKGROUND

During the late nineteenth and mid twentieth centuries, plants were among the sources of remedies for various infectious disease before improvements in synthetic chemistry and the unearthing of antimicrobials (Gwynn and Hylands, 2000). Human utilization of plants as traditional medication can be followed back to roughly 60,000 years (Solecki, 1975; Maridass and De Britto, 2008). These plants were utilized as flavors and spices, insect repellants and fumigants, ornamentals, cosmetics, foods and drugs (Kunin and Lawton, 1996; Pieroni *et al*., 2004; Maridass and De Britto, 2008).

In numerous underprivileged regions of the world, indigenous medication stays as one of the most inexpensive and effectively available type of treatment in the local health care systems. There is a long history on the traditional utilization of medicinal plants among the occupants of these regions. Notable confirmations among Indians and the Chinese on the utilization of specific herbs for remedial purposes goes back to 1600 - 3500 B.C. what's more, 4000 - 5000 B.C. respectively (Prakash and Gupta, 2005; Hosseinzadeh *et al*., 2015). Globally, medicinal plants constitute a major component of the indigenous medical system. The ethnobotany is basic for the research and development of natural drugs (Farnsworth, 1990). "Traditional" use of herbal medications indicates significant historic application which is without a doubt valid for different substances that are offered as "traditional herbal drugs". In some developing countries, a large part of the people rely upon conventional medicine specialists and their information on medicinal plants to meet wellness care needs. Albeit contemporary remedy may also exist together with such traditional standard, natural drugs have routinely kept up their prominence for cultural and ancient reasons (Vishwakarma *et al*., 2013).

It has been reported that about one quarter of medical prescriptions are formulations composed mainly of materials derived from vegetation or plant-derived synthetic analogs (Licciardi and Underwood, 2011). The World Health Organization (WHO), detailed that around 80 % of the world's people, normally the ones of developing nations rely upon plant-derived drugs for their

basic healthcare needs (Cragg *et al*., 1997; Hosseinzadeh *et al*., 2015). As indicated by the WHO, this "traditional medicine" includes the utilization of the know-how and practices of regular recovery for the inhibition, diagnosis, and elimination of physical, mental, or social unevenness (Akerele, 1993; Hosseinzadeh *et al*., 2015). Traditional medicine practitioners in several international locations, wherein conventional medication is as often as possible used to treat cut swelling, aging, asthma, cancer, diabetes, scabies, jaundice, skin infection, dermatitis, venereal diseases, wounds, snakebite, and gastric ulcer, give directions to local people regarding how to create medications from plants. Unfortunately, those practices preserved no records, and the records about natural drugs became fundamentally handed orally from one age group to the next (Dhar *et al*., 1968; Sofowora, 1996). The WHO has demonstrated splendid enthusiasm for archiving the utilization of medicinal plants by various tribes/communities in various areas of the world (Kaido *et al*., 1997). Many developing countries have increased their endeavors in reporting the ethnomedical records on medicinal plants. Research to find out logical proof for claims by tribal healers on Indian herbs was done. Those local ethnomedical mixtures were evaluated scientifically, appropriately circulated and individuals were educated with respect to strong medication treatment and improved well-being status (Manandhar, 1987).

Terrestrial plants, higher plants in particular, have been a critical component in battling different human illnesses. A considerable amount of species, including licorice (*Glycyrrhiza glabra*), poppy capsule latex (*Papaver somniferum*) and myrrh (*Commiphora* species) had been referred to by the main archived composed record on clay tablets from Mesopotamia in 2600 BC, and these plants are as yet being used today for the treatment of different ailments or as substances of drugs or natural preparations utilized in structures of traditional medication (Newman *et al*., 2000). Moreover, codeine, morphine, papaverine and noscapine (narcotine), secluded from *P. somniferum* have been processed as single chemical drugs and are still applied clinically (Newman *et al*., 2000; Newman *et al*., 2003; Chin *et al*., 2006). Aside all the records of these terrestrial plants and their importance in traditional medicine and natural drug research, these are also known to form intimate relationships with microorganisms in their environment, and these relationships range from mutualistic to parasitic. Plant-fungus and plant-bacterium interdependences in the terrestrial environment are very common and have been well studied (Crump and Koch, 2008).

Previous studies have shown the presence of symbiotic microorganisms (bacteria and fungi) in plants. These symbiotic microbes are known as endophytes (Nalini *et al*., 2014). These endophytes can produce a whole lot of bioactive compounds (Joseph and Mini Priya, 2011). Most of the past studies on natural drug were focused mainly on plants, but contemporary studies are looking more into the capacity of endophytes in producing novel bioactive compounds for feasible health and commercial applications (Pimentel *et al*., 2011).

1.2 JUSTIFICATION

The fight against numerous multi-drug resistant microorganisms requires extra active and advanced therapeutic agents. Of the over 250,000 medicinal plant species, just 5-15 % have been chemically and pharmacologically studied to decide their resourcefulness as therapeutic agents (Lahlou, 2007) (Lahlou, 2013). Additionally, the microbial sphere dominates 90 % of all-natural diversity, be that as it may, only <1% has been discovered or studied (Lahlou, 2013; Mathur and Hoskins, 2017). Known antibiotics are gradually becoming ineffective, hence the importance of the discovery of new ones. The exploration of antibiotic-producing bacteria is looking into more diverse environments, from marine sediments to mangroves and numerous potential leads have been identified (Manivasagan *et al*., 2014; Chamberlain, 2015).

Prior to the development of penicillin, the vast majority of the medication from natural products were sourced from terrestrial plants. With the great accomplishment of penicillin came an overwhelming interest in the use of microorganisms in drug discovery research. Terrestrial microorganisms are an abundant source of structurally diverse bioactive substances and have provided important contributions to the discovery of antibacterial agents including penicillin, cephalosporins, aminoglycosides, tetracyclines, polyketides, etc. (Dewick, 2002). Ongoing therapeutic use of metabolites of microbial origin have extended into cholesterol-lowering compounds (e.g., mevastatin and lovastatin), immunosuppressive compounds (e.g., rapamycin and cyclosporins), antidiabetic compound (e.g., acarbose), antihelmintic compound (e.g., ivermectin), and anticancer compounds (e.g., epirubicin, pentostatin and peplomycin) (Newman *et al*., 2003; Butler, 2005; Sneader, 2005; Chin *et al*., 2006).

Significant contributions have been recorded globally with the use of herbal merchandise in combating and prevention of human infections. Medications of natural origin are sourced from various materials which incorporate terrestrial plants and microorganisms, marine life forms, and terrestrial vertebrates and invertebrates (Newman *et al*., 2000), and their relevance in present day medication has been referenced in elite reports (Jones *et al*., 2006; Kingston, 2008). Lately, accounts on the use of traditional medicine and plant study has gotten more interesting (Newman and Cragg, 2007; Yuan *et al*., 2016).

A proportion of natural products from plants that are fundamental to modern medicine have been explored but, even so, the importance of preserving biodiversity around the world seems to be obvious (Chamberlain, 2015). The increasing demand for medicinal plants in herbal and traditional medication as well as in natural drug discovery research has become a threat to their existence (Shaban *et al*., 2016; Mathur and Hoskins, 2017).

The risk of the elimination of natural diversity on the bases of environmental complications, which may include, toxic waste (including toxic metals and cyanides) from synthetic compounds and industrial practices, global warming, and multidrug resistance and traditional drug treatments is currently overwhelming. Hence, it is expedient to establish the importance of common bioresources and natural biodiversity to impact research procedures in biotechnological and pharmaceutical organizations for use in the drug discovery process (Lahlou, 2013; Mathur and Hoskins, 2017).

The essential objective of all drug discovery exercises is to set up the most promising drug lead, that might be utilized as a therapeutic agent and improved for the treatment of clinical conditions, such as microbial infections, nervous system diseases, cancer, metabolic diseases and high blood pressure (Lahlou, 2013; Mathur and Hoskins, 2017).

Antibiotics, which are described as low-molecular-weight organic natural products produced using microorganisms that are bioactive at low concentration against different microorganisms (Demain, 1981), are the most biologically active natural products extracted from endophytes. The breakthrough of antibiotics came basically from when penicillin was discovered to the isolation

novel antibiotics from endophytes up to the year 2003 (Strobel and Daisy, 2003). A large number of these antibiotics have shown to be essential in disease control (Guo *et al*., 2008).

Solanum mauritianum, a plant used in South African traditional medicine for the treatment of various infections was selected for this study. This is based on the minimal work done on this plant species. While some of other *Solanum* species have been studied, very little information is found on *S. mauritianum* based on its biological activities, secondary metabolite profile and their associated microbial community, most especially, bacterial endophytes.

1.3 HYPOTHESIS

It was hypothesized in this study that:

1. *Solanum mauritianum* is a plant that is rich in bioactive phytochemicals and can be a source for possible drug leads to address the issue of antibacterial resistance.
2. Bacterial endophytes from *S. mauritianum* produce similar bioactive secondary metabolites and have similar biological activity as the plant.
3. If the plants and their resident microbes can produce similar secondary metabolites, the use of plants in drug discovery can be substituted with the use of their microbial symbionts to avoid over-harvesting plants and overexploiting our biodiversity.

1.4 AIM

This study aims to identify bacterial endophytes from *Solanum mauritianum*, extract their crude secondary metabolites and evaluate the antibacterial and anticancer activities of these crude secondary metabolites; as well as determine if the endophytes isolated are able to produce similar bioactive compounds as the plant and/or other promising bioactive compounds.

1.5 OBJECTIVES OF THE STUDY

To achieve the above-stated aim, the following specific objectives were set:

1. Isolation, characterization, and the identification of bacterial endophytes from *S. mauritianum*.
2. Extraction and identification of bioactive compounds from isolated endophytes of *S. mauritianum*.

3. Assessment of bioactivity of the secondary metabolites from bacterial endophytes of *Solanum mauritianum* against pathogenic bacteria and human cancer cells.

4. Extraction of *Solanum mauritianum* and identification of secondary metabolites present in plants as compared to the metabolites produced by endophytes.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MEDICINAL PLANTS AS TRADITIONAL MEDICINE

Human beings have always relied on nature as a source for drugs, housings, foodstuffs, scents, clothing, flavors, fertilizers, and way of transportation at some stage in the ages. For majority of the world's population, medicinal plants continue to show a dominant role in the healthcare system especially in developing countries, where herbal medicine has continuous history of long use. The development and recognition of both the medicinal and economic relevance of these plants have increased in both developing and industrialized nations (World Health Organization (WHO), 1998; Dar *et al*., 2017). Plants can offer mankind with new leads for medicines (Gurib-Fakim, 2006; Dar *et al*., 2017). In diversified industries, plants have remarkably contributed to the development of products such as pharmaceuticals and drugs, industrial raw materials, fine chemicals, cosmetics, etc. For new drug discovery and development, medicinal plants perform a dynamic part of providing a lead compound (Harrison, 1998; Jonas, 1998; Dar *et al*., 2017). These plants play essential roles in primary healthcare in many developing countries, inclusive of South Africa (Fullas, 2005). In these countries, due to affordability and cultural beliefs, about 80% of humans support the use of traditional medicines (Maroyi, 2013; van Wyk and Prinsloo, 2018). South Africa is reported to having in excess of 30,000 species of higher plants of which about 3000 could be utilized medicinally (van Wyk and Prinsloo, 2018).

According to history, herbs were utilized as poultices, tinctures, teas and powders; these herbs were later utilized as decoctions, and finally as pure compounds. Across different cultures, information about utilizing medicinal plants exists as oral lifestyle and local folk tales, which are accessible through families, clans, and cultures and these information passed down from generation to generation (Kumar *et al*., 2015). However, even in recent times, plants are not only essential in health care but are also the best source for safe medications (Hamburger and Hostettmann, 1991; Dar *et al*., 2017). Plants have been demonstrated to be sources of novel biologically active natural products as they have advanced and adapted over thousands of years to withstand microbes, bugs, parasites, and climate in order to supply various structurally unique secondary metabolites. Their

ethnopharmacological characteristics had been utilized as an essential source of medicine for early drug discovery (Fellows and Scofield, 1995; McRae *et al*., 2007; Kumar *et al*., 2015). Since days of yore, medicinal plants and their concentrates have provided significant medications, for example, antitussives (codeine), analgesics (morphine), cardiotonic (digoxin), antihypertensives (reserpine), antimalarials (quinine and artemisinin) and antineoplastics (vinblastine and taxol) which were utilized by people for specific illnesses (Ramawat *et al*., 2009; Kumar *et al*., 2015).

However, while we have available to us some several modern drugs, it is still unquestionably imperative to find and develop new curative agents. It has been approximated that only one-third of perceived human ailments have received acceptable therapy (Hamburger and Hostettmann, 1991). Hence, the battle against infections should be persistently continued. Traditional plant medicines still enjoy significant position in the modern-day drug industries due to their minor side effects as well as the synergistic action of their blend of compounds (Dar *et al*., 2017).

Present day exploration for bioactive compounds usually utilizes refined bioassays and bioassay-guided fractionation of medicinal herbs utilized by traditional healers. This has brought about the isolation of various new important therapeutic compounds. Numerous potent medications and a wide-reaching number of drug leads and novel pharmacologically active constituents have been created from herbal drugs because of the dedicated endeavors of scientists (Philipson, 1989; Dar *et al*., 2017). The production of morphine on an industrial scale by Merck in Germany in 1826 denoted the start of the commercialization of plant-based drugs (Grabley and Thiericke, 1999; Dar *et al*., 2017). It was reported that roughly half of the top-selling pharmaceuticals in 1991 were either natural products or their derivatives (Cragg *et al*., 1997; Dar *et al*., 2017). There is an ever-increasing need for new chemical compounds to treat infections among humans. The constant development of drug-resistant microorganisms, the latest discovery of cases of life-threatening infections, and the frequent reoccurrence of diseases have pushed for more innovations in the drug discovery field (Demain, 2000; Strobel *et al*., 2004).

2.2 NATURAL PRODUCTS

For many years, natural products have been reported as vital sources of active substances in therapeutic agents (Mathur and Hoskins, 2017). According to Mathur and Hoskins, (2017), “a

natural product refers to a chemical entity, formed by means of a naturally occurring living organism with pharmacological properties, which can contribute to vital drug discovery and design". The crude constituents extracted from medicinal plants, animals, microorganisms, or microbial fermentation broths comprise of unique, structurally varied chemical constituents. Natural products have been essential in the biotechnology and pharmaceutical industries on the premises that a range of new-age drugs are either from naturally occurring molecules, or their derivatives (Beattie *et al*., 2005; Atanasov *et al*., 2015; Mathur and Hoskins, 2017).

The role of natural products in treatment and prevention of diseases found in human cannot be over emphasized. An investigation of drugs discovered and developed between the years 1981 and 2002 uncovered that natural products or drugs from natural products account for 28% of most new chemical compounds discharged onto the market (Newman *et al*., 2003; Chin *et al*., 2006). Subsequently secondary metabolites from nature have been studied in living systems, they are consistently seen as demonstrating more "drug-likeness and biological friendliness than totally artificial molecules," (Koehn and Carter, 2005) making them the right component for additional drug development (Balunas and Kinghorn, 2005; Drahl *et al*., 2005; Chin *et al*., 2006; Jones *et al*., 2006).

It is important to mention that most innovative drugs were generated from natural products and from constituents of natural products (Lahlou, 2007). Natural products have remained a flourishing wellspring for the development of groundbreaking drugs by virtue of the assorted variety of their chemical components and their capacity to act on a wide range of biological targets (Bhutani and Gohil, 2010). Majority of natural products are compounds derived from primary metabolites for example, carbohydrates, amino acids, and fatty acids and are commonly categorized as secondary metabolites. Primary metabolites are a significant part of living organisms which are involved in the breakdown and biosynthesis of fats, proteins, carbohydrates and nucleic acids. Primary metabolites are directly involved in the metabolism and growth of cells. Then again, while secondary metabolites are produced as a result of primary metabolism, they are normally not involved in metabolic activities of the living organism. Examples of these secondary metabolites include: alkaloids, flavonoids, terpenes, sterols, lignins, phenolics, essential oils, tannins, and so forth (Ramawat *et al*., 2009). The system by which an organism biosynthesize its chemical

components, known as “secondary metabolites”, is frequently observed to be specific to that organism or is an expression of the uniqueness of a species and is alluded to as “secondary metabolism” (Maplestone *et al*., 1992). In this way, secondary metabolites are formed either because of the living organism adjusting to its environmental factors or as a plausible protection mechanism towards predators (Colegate and Molyneux, 2008; Kumar *et al*., 2015).

On a basic level, there are three pathways for discovering new pharmacologically noteworthy compounds: i. rational drug design, where the medication is intentionally customized towards explicit focuses in the microbial cell (Mandal *et al*., 2009); ii. combinatorial chemistry, which includes a blend of combinatorial library of compounds, which are then tried against the cellular focus to decide the most active compound (Gallop *et al*., 1994); and ultimately iii. natural product discovery, by extracting and isolating bioactive compounds from nature (Strohl, 2000). As of late, endeavors are being made to re-investigate the capability of natural products as wellsprings of novel medications (see figure 2.1).

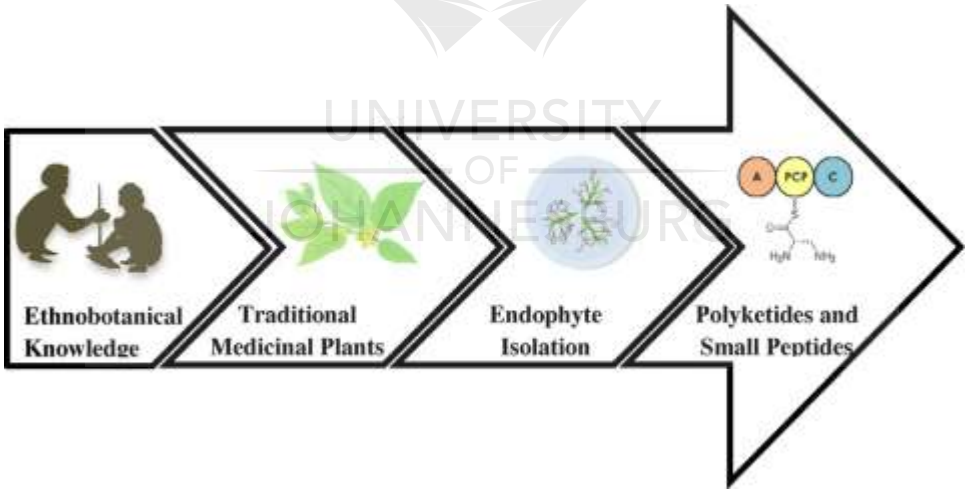


Figure 2.1: Graphical portrayal of natural product drug development approach (Alvin et al., 2014).

Medicinal plants are of significant importance to the healthcare of people and communities. The therapeutic benefits of these plants is from a couple of chemical compounds that produce particular physiological activity on the human body. The most significant of these bioactive constituents of plants are alkaloids, flavonoids, tannins, and phenolic compounds (Edeoga *et al*., 2005).

2.2.1 Phytochemicals: A Brief Overview

The curative capacities of chemical compounds found in nature: "phytochemicals" in plants have been reported in various investigations (Dillard and German, 2000; Vauzour *et al*., 2010; Ruiz and Hernández, 2016). As non-nutritive constituents of plants, phytochemicals as per past examinations display diverse remedial features especially in the control of various diseases such as diabetes, cancer, erectile dysfunction, cardiovascular disease, etc. These therapeutic attributes of phytochemicals are accounted for to be because of the interactions with the various biological structures comprising of the endocrine and hormonal frameworks. They are generally dispersed in, vegetables, fruits, whole grains, legumes, seeds, nuts, herbs and spices and in beverages that are plant-based which includes tea and wine and might be arranged according on their chemical structure (Oboh *et al*., 2018).

There are reports of phytochemicals being able to inhibit microorganisms by way of interfering with signal transduction pathways as well as some metabolic processes or modulating gene expression (Kris-Etherton *et al*., 2002; Manson, 2003; Surh, 2003). As far as application, phytochemicals may either be utilized as chemotherapeutic or chemopreventive metabolites. There are three major classes of chemicals synthesized by plants, they include: terpenoids, phenolic compounds, and alkaloids and other nitrogen-containing plant metabolites (Dillard and German, 2000)

2.2.2 Classes of phytochemicals

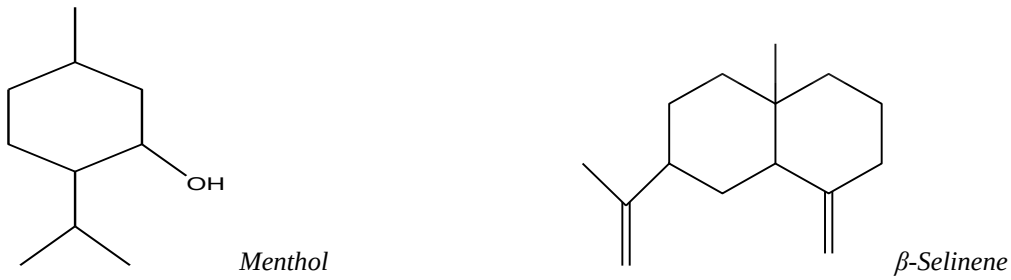
Phytochemicals are chemical compounds found in plants that generally protect such plants against pathogenic microbes that affect plants. The expression "phytochemical" addresses an assortment of metabolites synthesized by plants, nevertheless, is explicitly used to indicate those metabolites that may have an impact on human health (Koche *et al*., 2016). Many phytochemicals have been distinguished by scientists, albeit just a couple have been firmly examined. Phytochemicals have shown a few physiological activities (Edeoga *et al*., 2005; Peteros and Uy, 2010; Ogunmefun, 2018). A few of the phytochemicals that are in existence and their helpfulness are shown on table 2.1.

Table 2.1: Occurrence and job of significant classes of phytochemicals (Koche *et al*., 2016).

Class of phytochemical	Occurrence as natural product (%)	Role in health care
Phenolics	45	Antioxidants, anti-cancerous, cytotoxicants, anti-microbials and vasodilating
Terpenoids and Steroids	27	Anti-microbials, detoxifying agents, strengthners, anti-rheumatics, anti-malarial, hepaticidal
Alkaloids	18	Neuropharmaceuticals, anti-cancerous, sedatives, anti-microbials, insecticidal
Other chemicals	10	Anti-inflammatory, Immunostimulating

2.2.2.1 Terpenes

Terpenes are hydrocarbons (figure 2.2) comprised of compounds from nature derived from five-carbon isoprene. Terpenes are polymers of five-carbon hydrocarbon isoprene and are various lipids found in every single living organism and natural product (Morrison and Boyd, 2006). Their isoprene (5C) unit is nature's preferred building block. Terpenes have numerous isoprene units appended in a customary head to tail style (Brady and Trams, 1964). Numerous flavorings and pleasant aromas are comprising of terpenes because of its top-notch smell. Terpenes and its subsidiaries are applied as antimalarial medications, for example, artemisinin and related compounds (Perveen, 2016).



Menthol *β-Selinene*

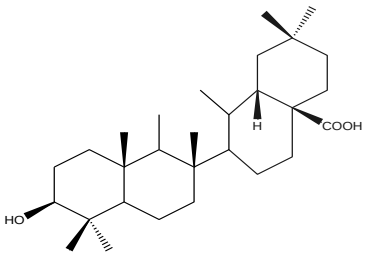
Figure 2.2: Structures of terpenoids

2.2.2.1.1 Saponins

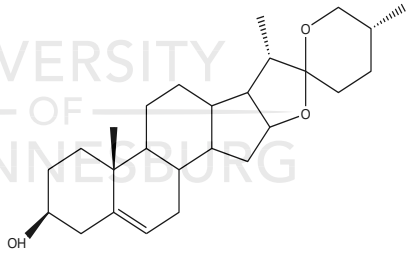
Saponins are a class of phytochemicals present in large quantities in various types of plants. They are explicitly amphipathic glycosides and are organized phenomenologically by their forming soap-

like foaming when shaken in aqueous solution (Hostettmann and Marston, 2008; Koche *et al*., 2016). They are arranged structurally by their ownership of at least one hydrophilic glycoside moieties joined with a lipophilic triterpene derivative (Hostettmann and Marston, 2008). Two significant sets of saponins exist which are steroid and triterpene saponins. Saponins are insoluble in ether however dissolvable in water, and on hydrolysis, they give aglycones like glycosides. They cause hemolysis of blood and are poisonous to cattle as they are incredibly toxic (Kar, 2007). Aside from irritating the mucous membranes, they have a sour and bitter taste. They are dissolvable in water and alcohol however in solvents like benzene and n-hexane that are natural and nonpolar, they are insoluble; consequently, they are for the most part amorphous in nature. Saponins are remedially significant in light of the fact that they cholesterol in the body (hypolipidemic) and have anticancer potentials. Saponins work synergistically with cardiac glycosides (Sarker and Nahar, 2007).

Saponins are known to be anti-inflammatory (Just *et al*., 1998). The ability of saponins in lowering cholesterol in the long run helps to diminish the danger of cardiovascular diseases, for example, hypertension which as a rule prompts stroke (Francis *et al*., 2002). Structures of two realized saponins are found in figure 2.3.



Oleanolic acid



Diosgenin acid

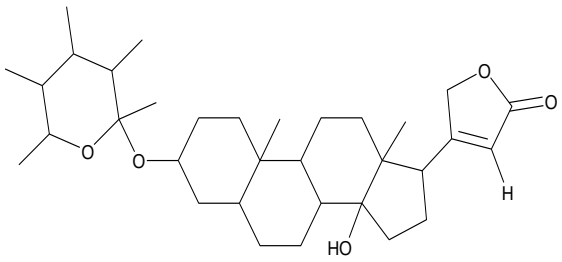
Figure 2.3: Structures of saponins

2.2.2.1.2 Glycosides

Glycosides are the buildup results of sugars (including polysaccharides) with various assortments of organic hydroxyl (thiol occasionally) compounds. Glycosides are colorless crystalline plant constituents that are soluble in water and present in the cell sap of plants. Glycosides chemically composed of a non-carbohydrate part (aglycones or genin) and a carbohydrate (glucose) (Kar, 2007; Firn, 2010). Glycosides can be promptly hydrolyzed into its constituents with mineral acids as they react neutrally and are bitter essence regularly found in plants of the family Genitiaceae. The bitter

action on the gustatory nerves brings about increased salivation and flow of gastric juices (Kar, 2007; Firn, 2010). Sometimes, due to the presence of tannic acid, some of the bitter essence are either utilized as astringents (control the flow of blood), to regulate cell metabolism and growth by reducing thyroxin, or as antiprotozoal. Some examples of glycosides are cardiac glycosides (see figure 2.4) which act on the heart and anthracene glycosides that function as laxative and for treatment of skin infection, while chalcone glycosides are utilized as anticancer agents (Sarker and Nahar, 2007). The constituents of plants containing cyanogenic glycosides have been accounted for to being helpful as flavor enhancers in numerous pharmaceutical preparations. Amygdalin has been utilized in the treatment of cancer (hydrogen cyanide (HCN) freed in stomach kills malignant cells) and as a cough suppressant in different formulations. Consumption of cyanogenetic glycosides in large amounts can be risky (Sarker and Nahar, 2007).

Plants since long ago are utilized as arrow poisons (e.g., Strophanthus) or as heart drugs, for instance, digitalis, contain heart cardiotonic glycosides (Dewick, 2002). Cardiac glycosides help in the treatment of congestive heart failure; that is, they help a debilitated heart to be reinforced and work all the more effectively (Colin and Shelia, 2001). Cyanogenic glycosides are a collection of materials mostly derived from plants which on hydrolysis release hydrocyanic acid. Hence they call for consideration and an object of concern on account of their capacity in causing harm by poisoning as they are common toxins. For instance, Cassava, a food plant (Manihot esculenta), produces cyanogenic glycosides which require lengthy hydrolysis and boiling when preparing the tuberous roots to discharge and remove the HCN before it is consumed (Odugbemi, 2008).

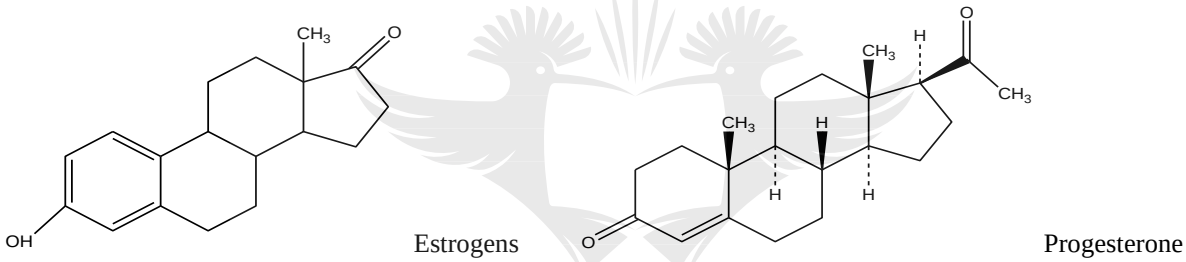


Cardenolides

Figure 2.4: Structure of glycoside

2.2.2.1.3 Steroids/Plant sterols

Plant steroids are one of the most naturally occurring phytoconstituents that are used remedially as cardiovascular medications or arrow poisons (Firn, 2010). The cardiac glycosides are mostly steroids having a characteristic capacity to employ explicit and strong activity on the cardiovascular muscles predominantly when infused into man or animals. Steroids (anabolic steroids) can advance nitrogen retention in osteoporosis (an infection following menopause in females making bones be permeable and thus stand more risk of fracture) and in creatures with wasting ailment (Maurya *et al*., 2008; Madziga *et al*., 2010). Steroids can display biological activities, for example, antifungal, antiviral, antileukemic, hypnotic, antipyretic, and muscle-relaxant activities and are found in huge amounts in numerous plants (Kokpol *et al*., 1986). Figure 2.5 shows the structures of two known steroids.



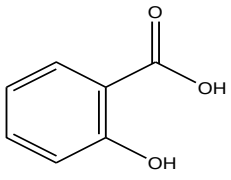
Estrogens Progesterone

Figure 2.5: Structures of steroids

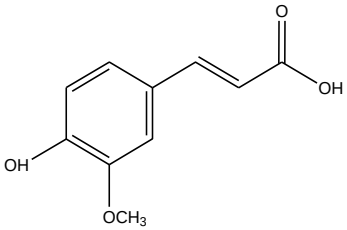
2.2.2.2 Phenols

Phenols act as antioxidants and contribute to the control of different degenerative diseases (Rice-Evans *et al*., 1996). Phenols, phenolics, or polyphenolics are chemical compounds that are usually present as natural color pigments that is responsible for the colors of plants' fruits. The activity of phenyl-alanine ammonia lyses (PAL) on phenylalanine prompts the blend of phenolics in plants. Among the different activities of phenols, it offers plants protection against microbes and predators of herbivores and are also utilized in the control of human diseases brought about by microorganisms (Puupponen-Pimiä *et al*., 2005). Caffeic acid (see figure 2.6), is the most well-known of phenolic compounds boundless in plants, which is trailed by chlorogenic acid, the causative agent of excessive irritation of the skin (dermatitis) in humans (Kar, 2007). Phenolics are natural antioxidants that work as nutraceuticals and present in apples, red wine and green tea. They

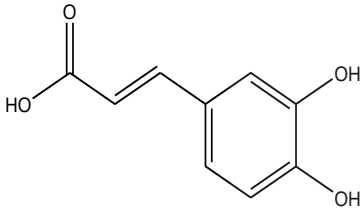
are also anti-inflammatory and anticancer agents and also prevent heart diseases (Ogunmefun, 2018).



Salicylic acid



Ferulic acid



Caffeic acid

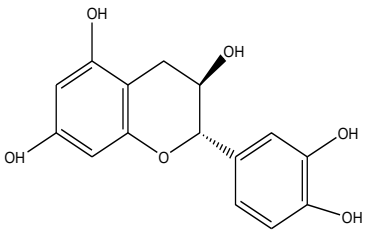
Figure 2.6: Structures of phenolic compounds

2.2.2.2.1 Tannins

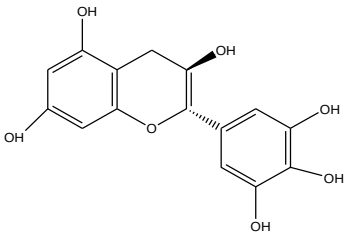
Tannins metabolites which are found principally in many plant species which helps to protect these plants against predators and pests; as well as contribute to plant development (Kathie *et al*., 2006). Tannins are astringent and are responsible for the dry and puckery feeling in the mouth in the wake of eating unripe fruits or drinking red wine (McGee, 2006). Ripening of fruits and aging of wine is also a result of the modification and/or the destruction of tannins over time. They are acidic and this acidity is credited to the presence of carboxylic groups or phenolics (Kar, 2007). Tannins form complexes with alkaloids, carbohydrates, proteins and gelatin (Ogunmefun, 2018).

Tannins are grouped into hydrolysable tannins and condensed tannins. Hydrolysable tannins, are the ones that produce gallic and ellagic acids when hydrolyzed, and based on the kind of acid produced, the hydrolysable tannins are either called gallotannins or ellagitannins; furthermore, when heat is applied to tannins, they form pyrogallic acid (Kathie *et al*., 2006). The usefulness of tannins as antiseptic is conferred on them by the presence of phenolic groups (Kar, 2007). Examples of hydrolysable tannins are: genistein, daidzein, glycitein and aflavins (from tea). Plants that contain tannins have been accounted for to be astringent in nature and are helpful in the treatment of gastrointestinal ailments like dysentery and diarrhea; implying tannins have antimicrobial activities

(Scalbert, 1991; Dharmananda, 2003). Tannins also possess strong antioxidant properties (Harborne, 1998; Trease and Evans, 2009; Ogunmefun, 2018).



Catechin

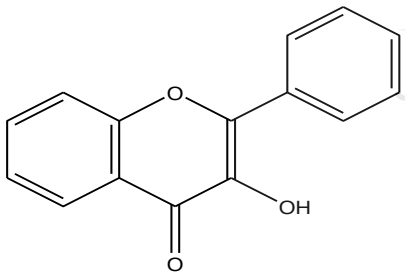


Gallocatechin

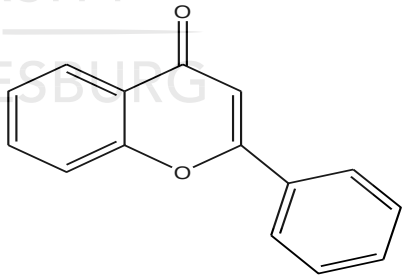
Figure 2.7 : Structures of tannins

2.2.2.2.2 Flavonoids

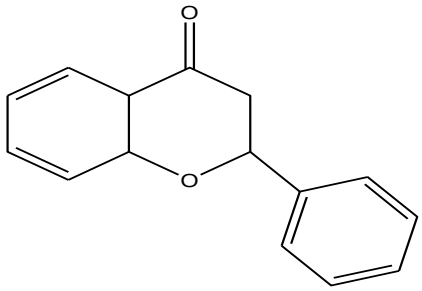
Flavonoids are a significant part of polyphenols which are broadly distributed among plants. The flavonoid structure contains more than one benzene ring (see figure 2.8 for some flavonoid structures) and they are utilized as free radical scavengers and as antioxidants (Kar, 2007). Flavonoids sometimes exist as pigments in higher plants. About 70% of plants contain flavonoids, like quercetin, quercitrin and kaempferol. Among the various groups of flavonoids that exist are flavans, flavones, flavonols, dihydroflavons, chalcones, anthocyanidins, and catechin (Kar, 2007). Flavonoids have the ability to help reduce coronary heart diseases (Rice-Evans *et al*., 1996) and have anti-inflammatory, anticoagulant, and aphrodisiac properties (Houghton *et al*., 2005).



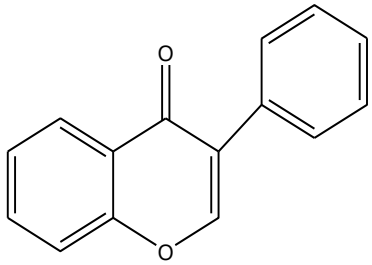
Flavonol



Flavone



Flavanone



Isoflavone

Figure 2.8: Structures of flavonoids.

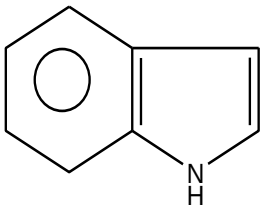
2.2.2.3 Nitrogen Containing Phytochemicals

2.2.2.3.1 Alkaloids

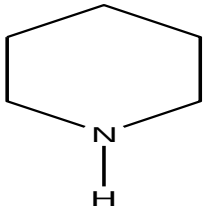
Alkaloids are the major group of secondary metabolites comprising of ammonia made up of nitrogen bases synthesized from building blocks of amino acid with different radicals substituting one or more hydrogen atoms in the peptide ring (see figure 2.9), containing for the most part oxygen (Firn, 2010). The compounds are basic in nature and turn red litmus paper blue, showing their alkalinity. One or more of the nitrogen molecules that are available in an alkaloid (normally as 1°, 2°, or 3° amines) add to the basicity of the alkaloid. The level of basicity shifts significantly, contingent upon the structure of the atom, and the presence and location of the functional groups (Sarker and Nahar, 2007). They react with acids to frame crystalline salts without the water as a by-product (Firn, 2010).

Alkaloids are for the most part promptly dissolvable in alcohol yet are slightly soluble in water however their salts are normally dissolvable in water. Alkaloidal solutions are usually bitter and alkaloids guard plants against herbivores and plant pathogens and are utilized broadly as pharmaceuticals, narcotics, stimulants and poisons as a result of their biological potencies (Madziga *et al*., 2010). Alkaloids are found in enormous amounts in seeds and plant roots and usually in combination with vegetable acids. Alkaloids are helpful in pharmacological applications as central nervous system (CNS) stimulants and sedatives (Madziga *et al*., 2010).

Alkaloids additionally are very helpful as pain relievers (Kokate *et al*., 2002). Atropine is an alkaloid utilized generally in medication as an antitoxin to organophosphate poisoning, while caffeine animates CNS and respiratory frameworks (Chapman and Mickleborough, 2009) (Eddleston and Chowdhury, 2015). Caffeine likewise fills in as an antidote to barbiturate and morphine poisoning, while emetine from Cephaelis ipecacuanha root is valuable in the treatment of protozoal infections, like, amoebic dysentery (Ogunmefun, 2018).



Indole alkaloid nucleus



Piperidine alkaloid nucleus

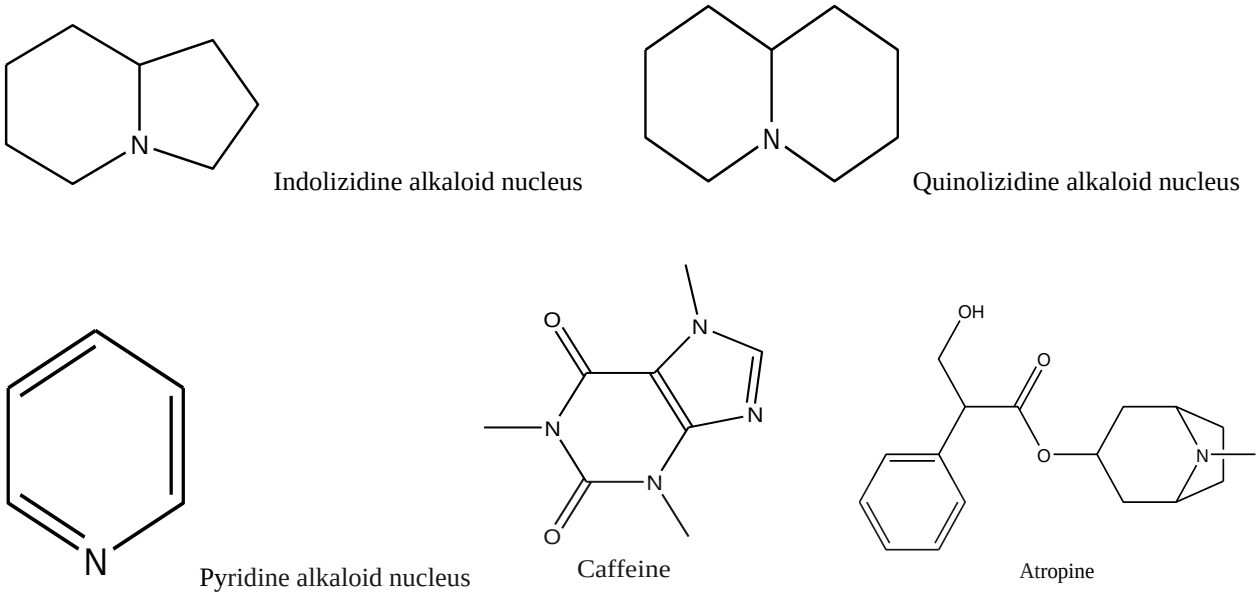


Figure 2.9: Structures of different alkaloids.

2.3 FAMILY SOLANACEAE: OVERVIEW

The Solanaceae (nightshades) is an important plant family with huge financial significance attached to it (Hawkes, 1999). A vast concentration of diversity of the Solanaceae is seen mostly in South America where it is recognized to have originated from (Hunziker, 1979). The plant family is represented on every vegetated continent based on its spread from the neotropical area. Besides their wide scope of different uses (for example traditional medication, cultural practices, ornamental horticulture, pharmacology, etc.), plants from the Solanaceae family have gigantic worldwide importance as food plants (Samuels, 2009; Samuels, 2015). The Solanaceae is an enormous varied family of trees, shrubs and herbs that is made up of 90 genera and over 2000 species. Solanaceae are known for having a wide range of alkaloids (Shah *et al*., 2013).

The Solanaceae are additionally the third most economically significant plant taxon and the most important as far as vegetable yields, and are the most varying of crops species on the basis of agricultural utility, as some of the crops include: various fruit-bearing vegetables (Tomato, Eggplant, Peppers), the tuber-bearing potato, ornamental plants (*Petunias, Nicotiana*), plants with eatable leaves (*Solanum aethiopicum*, *S. macrocarpon*) and medicinal plants (e.g. *Datura*, *Capsicum*) (Jagatheeswari, 2015).

Regardless of their extraordinary lushness of these species, they are not evenly dispersed between the genera. The eight most significant genera contain over 60% of the species (Griffin and Lin, 2000).The most financially feasible variety of the Solanaceae family is *Solanum*, which includes tomato (*Solanum lycopersicum*), the potato (*Solanum tuberosum*), and the aubergine or eggplant (*Solanum melongena*). Another significant genus is *Capsicum* which produces both bell and chili peppers (Jagatheeswari, 2015).

Medically significant species of the family Solanaceae are of the following genera: *Solanum*, *Datura*, *Capsicum*, *Nicotiana*, *Atropa*, *Withania*, *Hyoscymus*. These species are widely utilized for therapeutic reason all through different part of the world (Shah *et al*., 2013).

2.4 THE GENUS *SOLANUM*

Solanum, the nightshade is one of the biggest and hyper varied class of the family Solanaceae. Its hyper assorted variety not just makes it fascinating according to the taxonomic perspective but also for its helpfulness to humankind (Shah *et al*., 2013). They develop as vines, forbs, shrubs, subshrubs, and small trees, and usually have alluring fruits and flowers. The genus these days contains about 1,500 to 2,000 species. The species from this genus are being utilized for therapeutic reason from ancient time. Most parts of *Solanum* plants, particularly the unripe fruits and green parts, are toxic to humans however numerous species in the genus bear some eatable parts, for example, tubers, fruits or leaves (Shah *et al*., 2013). Some species are cultivated, and they include three universally significant food crops:

Tomato, *S. lycopersicum*

Potato, *S. tuberosum*

Eggplant, *S. melongena*

2.5 THE SPECIE *SOLANUM MAURITIANUM*

Native to many South American nations, *Solanum mauritianum* Scopoli has become invasive to tropical, subtropical and warm climate regions of the world, prominently in New Zealand, Australia, Madagascar and South Africa (Olckers, 1999; Olckers, 2011; Cowie *et al*., 2018; English and Olckers, 2018). Generally known as bugweed or woolly nightshade, this small tree that is fast-growing mostly invades conservation areas, predominantly in high-rainfall areas, forestry

plantations, agricultural land, and riparian zones (Olckers, 1999; Olckers, 2011; Cowie *et al*., 2018; English and Olckers, 2018). Invasion is encouraged by flowers blossoming all year round (Henderson, 2001) and the production of various berries which attract frugivorous birds as agents of seed dispersal (Jordaan and Downs, 2012; Mokotjomela *et al*., 2013; English and Olckers, 2018).

2.5.1 Botanical description of *S. mauritianum*

Solanum mauritianum is a small, perennial tree with broad leaves, that typically grows up to four meters in height. The leaves and stems are shrouded in pubescent, felt-like hairs which cause respiratory and dermal irritation, making the plant profoundly unpalatable to domestic animals or wild game (Olckers, 2009). The unripe fruits are known to be rich in alkaloids, namely solasodine, making the plant poisonous to humans and animals alike (Henderson, 2001), barring frugivorous species which feed on ripe fruits. When mature, *S. mauritianum* flowers and fruits bloom constantly and produce ample amount of seeds (Witkowski and Garner, 2008), which are the tree's main reproductive route. Furthermore, with its high production of seeds and fruits, *S. mauritianum* is self-reliant (Cowie *et al*., 2017) and can redevelop quickly after felling (Witkowski and Garner, 2008). The invasion of weeds is enabled by huge seed banks (Goodall *et al*., 2017) and dispersal is done by frugivorous birds and mammals, which promptly feed on the plentiful fruits, while scattering the seeds over long distances (Jordaan *et al*., 2011; Cowie *et al*., 2018).

Figure 2.10: Ariel parts of Solanum mauritianum.

2.5.2 Global Distribution of *S. mauritianum*

Solanum mauritianum Scopoli is an invasive tree of worldwide noteworthiness. Reported to have originated from South America, especially southern Brazil, north-eastern Argentina, Uruguay and Paraguay (Roe, 1972; Cowie *et al*., 2018). The tree was deliberately introduced as ornamental plants and inadvertently spread by maritime colonists, into various warm temperate, tropical and sub-tropical nations and islands around the world. *Solanum mauritianum* has naturalized in many nations around the world, accomplishing invasive status in South Africa, Swaziland, Uganda Australia, New Zealand, the Cook Islands, Fiji, India, Madagascar, Kenya, New Caledonia, Papua New Guinea, Sri Lanka, St Helena, and parts of the United States, including Hawaii, California and Florida. Be that as it may, the most intense invasion has happened in South Africa, where the tree presently holds in excess of 80,500 ha of land, mostly all through the eastern higher rainfall areas of the nation (Henderson, 2001; Marais *et al*., 2004; Cowie *et al*., 2018).

Able to grow in an assortment of soil types and environments, *S. mauritianum* invades urban and peri-urban rural areas, riparian zones, savannas, prairies, rail-and street sides, woods edges, farmlands, regular timberlands and business ranger service ranches (Olckers, 1999; Henderson, 2001; Cowie *et al*., 2018). The invasion of *S. mauritianum* regularly brings about the development of impenetrable stands, which outcompete and conceal surrounding vegetation (Henderson, 2001; Cowie *et al*., 2018).

2.5.3 Ethnobotany of *Solanum mauritianum*

Over 30,000 plant species are native to South Africa and more than 3,000 of those are utilized in traditional medicine practices around the country (van Wyk *et al*., 1997). *S. mauritianum* (uMbangabanga in Xhosa) (Dold and Cocks, 2001), despite the fact that it is viewed as an invasive weed, is utilized by traditional healers in South Africa and other African nations and a few countries the world over to treat signs and manifestations of various diseases. In Table 2.2, the various traditional uses of *S. mauritianum* medicinally are highlighted.

Table 2.2 below summarizes the ethnobotanical uses of *S. mauritianum*.

Part(s) used	Administration	Method of preparation	Traditional uses/Therapeutic uses	Country	References
Bark	Taken orally as an infusion: half a glass to one glass 3 times a day for 10 days to one and half months	Bark powder is boiled in water	Anti-cancer (Colorectal cancer)	Kenya	(Ochwang'i *et al*., 2014)
Bark	Taken after meals	Usually boiled together with *Prunus africana* (Hook.f.) Kalkman stem bark and roots.	Causes one to have increased appetite	Kenya	(Ochwang'i *et al*., 2014)
NS	NS	NS	It is also used to treat Skin rashes	Kenya	(Njoroge *et al*., 2004)
Roots	NS	NS	Used in the treatment of menorrhagia	South Africa	(Hutchings *et al*., 1996; Lewu and Afolayan, 2009; Ochwang'i *et al*., 2014)
Leaf	NS	NS	Used to treat infertility and menorrhagia	South Africa	(Mabogo, 1990)
Bark	NS	NS	Treat abdominal pains	Madagascar	(Rabearivony *et al*., 2015)
Leaf	NS	NS	Infusion is used to treat dysentery and diarrhoea	South Africa	(Watt and Breyer-Brandwijk, 1962; Lewu and Afolayan, 2009)
Fruits	NS	Chopped and macerated in warm water for 24 h and one tin cup of the decoction is administered by healer anally via fatal bulb syringe. Thrice week	Cleaning kidneys	South Africa	(Jäger *et al*., 1996; Semenya *et al*., 2012)

Part(s) used	Administration	Method of preparation	Traditional uses/Therapeutic uses	Country	References
Roots	NS	Two spoons-full of grated root are boiled for ten minutes and given to cows (750 ml every second day)	To treat dystocia.	NS	(Dold and Cocks, 2001)
Roots	NS	Handful of roots are boiled with 2 L water for 10–15 min. Full cup (300 mL) is taken twice a day orally	Used to treat Sexually transmitted infections (STIs): Gonorrhea, Chlamydia, Syphilis,	South Africa	(Maema *et al*., 2019)
Roots	Oral	NS	Treat stomach aches	South Africa	(Mongalo and Makhafola, 2018)
Leaves	NS	NS	Mosquito repellent	Australia	(Packer *et al*., 2012)
Leaves	NS	NS	Snuffed	South Africa	(Lindsey *et al*., 1999)
NS	NS	NS	Ophthalmic, ear, nose and throat ailments. Eye problems, earaches	South Africa	(Mhlongo and Van Wyk, 2019)

NS = Not specified, SA

2.5.4 Phytochemistry of *Solanum mauritianum*.

Solanum species are a one of a kind wellspring of many pharmacologically essential lead compounds, particularly steroidal alkaloids like the solasodine, solasonine, solamargine and assortments of so many other therapeutically valuable alkaloids. *Solanum mauritianum* remarkably contains exorbitant concentrations of alkaloids. The alkaloid solasodine (which is most concentrated in the unripe green berry), solauricine, solauricidine, and solasodamine are some of the toxic phytochemicals present in *S. mauritianum* (Ochwang'i *et al*., 2014). Alkaloids, glycosides, flavonoids, phenols, tannins, and saponins are all phytochemicals that have been reported present in *Solanum mauritianum* (Chaitanya *et al*., 2015; Jayakumar and Murugan, 2016a). Table 2.3 as adapted from (Jayakumar and Murugan, 2016b) offers a concise summary of the major *Solanum* alkaloids.

Table 2.3: Derivatives of *Solanum* alkaloids and its therapeutic significance.

Alkaloid	Derivative	Activity
Solanidine	Solanidine N-oxide; 5 alpha, 6-dihydrosolanidine	Teratogenicity
Solasodine	Solasodine O-(diethyl phosphate); N-acetyltetrahydrosolasodine	Cholinergic
Tomatidine	Dihydrotomatidine; Pregnane derivative	Toxicity study, neuritogenic and NGF-enhancing activities.
Solamargine	(25R)-3b-{O-a-L-rhamnopyranosyl-(1®2)- [O-a-L-rhamnopyranosyl-(1®4)]-b-D-glucopyranosyloxy}-22a-N-spirosol-5-ene	Anticancer
Solanopubamine	3-βN, 23-βO-diacetylsolanopubamine; 3, 3-βN,βN-dimethyl-βO-methylsolanopubamine; 3-βN-octadecanoly-solanopubamine; solanopubamine-23-βO-octadecanoate; Solanopubamine 23-βO-undec-11-enoate; Solanopubamine-23-βO-acetate	Anticancer and antimicrobial
Chaconine	6-O-sulfated chaconine	Cytotoxicity studies
Solanine	6-O-sulfated solanine	Cytotoxicity studies

2.5.5 Biological Activities of *Solanum mauritianum*

S. mauritianum leaves have been mentioned to have prostaglandin synthesis inhibition (Jäger *et al*., 1996; Lindsey *et al*., 1999). The plant is understood to contain solasodine (Drewes and van Staden, 1995), a compound with recognized anti-inflammatory activity (Bhattacharya *et al*., 1980; Lewis, 1989). *Solanum* species are reported to have antibacterial and antifungal properties against various pathogenic organisms (Niño *et al*., 2006). Fewell *et al*. (1994) reported the inhibition of the development of mycelium in *Phoma medicaginis* and *Rhizoctonia solani* by solamargine and solasonine which are known glycoalkaloids from *Solanum* species. A combination of 50 μM of each of the two glycoalkaloids delivered synergistic impacts against both fungal species, especially *R. solani*, which was basically unaffected by either compound, however, was notably inhibited by a 1:1 combination of the two glycoalkaloids. Solamargine was responsible for a 50 % growth inhibition of *P. medicaginis* at 60 μM (pH 7), germination of spores in *Alternaria brassicicola* was also notably inhibited by 100 μM solamargine (Singh and Singh, 2010).

Lupeol, apigenin, and solamargine that are secondary metabolites from *Solanum* species have been reported to exhibit anticancer property (Kuo *et al*., 2000; Chaturvedi *et al*., 2008; Siddique *et al*., 2008). Solamargine is believed to have induced cellular death by apoptosis based on the appearance of solamargine-treated cells of chromatin condensation, DNA fragmentation, and a sub-G1 peak in a DNA histogram. The highest number of dead Hep3B cells was observed within 2 hours of incubation with solamargine at constant concentrations, and additional cell death was not observed after an extended incubation with solamargine, indicating that the action of the solamargine could not be reversed (Singh and Singh, 2010).

2.6. PLANT AND MICROORGANISM RELATIONSHIPS

2.6.1 Symbiosis of plants and microorganisms

Plant-microorganism beneficial interaction has noteworthy significance in view of its critical job in cycling of nutrients. Microsymbionts of this relationship, organisms, show a few significant plant development advancing highlights (Kloepper *et al*., 1999; Compant *et al*., 2005; Gray and Smith, 2005; Tariq *et al*., 2017).

Associative microsymbionts for the most part live in the rhizosphere, rhizoplane and phyllosphere or sometimes live inside the plant tissue and organ. Microorganisms associated with rhizosphere and rhizoplane are named as rhizobacteria, while those carefully found inside the plant are known as endophytic microscopic organisms (Tariq *et al*., 2014; Tariq *et al*., 2017).

Microsymbionts attach themselves with to the plant roots through cell surface polysaccharides or cell structures including fimbriae and flagella. Microbes, cyanobacteria, parasites and green growth live in and around the root zone however microorganisms is the most bountiful (Saharan and Nehra, 2011; Tariq *et al*., 2017). The associative microsymbionts set up a relationship with plant for supplement and shelter and consequently advance plant growth mostly by fixing nitrogen, indole phosphate solubilization, acetic acid production and biocontrol of plant diseases (Tariq *et al*., 2017).

2.7. BENEFICIAL PLANT BACTERIA/FUNGI

2.7.1 Root colonizing microscopic organisms

Various microorganisms live in the segment of soil altered or affected by plant roots, which is the alleged "rhizosphere" (Kent and Triplett, 2002; Selosse *et al*., 2004). Among these microorganisms, some positively affect plant growth, and they are referred to as "plant growth-promoting rhizobacteria" (PGPR), for example, *Azospirillum*, *Pseudomonas*, Agrobacteria, a good number of Gram-positive *Bacillus*, etc. (Kapulnik, 1991; Selosse *et al*., 2004). PGPR are engaged in fixing free N2, and they likely synthesize vitamins and phytohormones (Sturz *et al*., 2000; Selosse *et al*., 2004). When compared to mycorrhizae or nodules, the role of PGPR have just recently been recognized and they are reported to also contribute to the protection of plants. They also provide valuable experimental models for genetic research (which has remained inadequately developed for explaining the mechanisms employed by mycorrhizal fungi for plant protection) and have been frequently used for rhizospheric microbes, most especially bacteria, to distinguish critical genes (Selosse *et al*., 2004).

In addition to roots and soil, microbial symbionts also occur in all other plant tissues. In the last decade, a large group of endophytic microflora, such as, bacteria and fungi were isolated from various plants without symptom of disease using the DNA-based approach (Redlin and Carris, 1996; Saikkonen *et al*., 1998; Sturz *et al*., 2000; Weidner *et al*., 2000; Garbeva *et al*., 2001; Yang

et al., 2001; Girlanda *et al*., 2002; Arnold *et al*., 2003). These microflora includes latent pathogens (Ahlholm *et al*., 2002), saprotrophs (Jumpponen and Trappe, 1998) and occasionally contaminants, but additionally symbionts (Saikkonen *et al*., 1998).

2.7.2 Endophytes

A wider range of microbial communities known as endophytes are housed inside various plants (Hallmann *et al*., 1997; Tian *et al*., 2017). It is broadly acknowledged that as universal colonizers of plants, endophytes have an effect on general plant wellbeing and profitability (Turner *et al*., 2013; Tian *et al*., 2017). Additionally, some of the advantageous impacts of endophytes on their host plants are seen through heaps of mechanisms including, advancing plant growth by means of the suppressing plant pathogenic organisms; synthesizing antimicrobial substances; increasing the competition for space, nutrients and ecological niches and producing biostimulants, such as phytohormones and peptides, which have no side effects on the customer, user or the habitat (Rosenblueth and Martínez-Romero, 2006; Bulgarelli *et al*., 2013; Gaiero *et al*., 2013; Turner *et al*., 2013; Tian *et al*., 2017).

Endophytes are microorganisms dwelling in the interior tissues of the plants without perpetrating observable signs and symptoms (Stone *et al*., 2000; Nisa *et al*., 2015). Most endophytes spend the whole or a part of their life cycle inside plant tissues (Wilson, 1995; Azevedo *et al*., 2000; Bacon and White, 2000; Saikkonen *et al*., 2004; Eljounaidi *et al*., 2016). The expression "Endophyte" was first presented by De Bary in 1866 (De Bary, 1866; Nisa *et al*., 2015) and was at first applied to any life form found inside a plant that causes asymptomatic infections totally inside plant tissues with no side effects of an infection (Wilson, 1995; Nisa *et al*., 2015). Endophytes are known to biosynthesize chemical compounds that are alike to those synthesized by host plants, perhaps as an adjustment to their host's microenvironment (Zhang *et al*., 2006; Martinez-Klimova *et al*., 2017).

These endophytes live in the intercellular regions of stems, petioles, roots, and leaves of plants bringing about no noticeable sign of their presence and have basically gone unnoticed (Strobel and Long, 1998; Nisa *et al*., 2015). The beneficial interaction that exists between plant and endophyte, that is, the plant feeds and protects the endophytes which produces 'consequently' bioactive (antibacterial, antifungal, antiviral, plant growth regulatory, insecticidal, etc.) materials to help the

development and competitiveness of the host in nature (Carroll, 1988; Nisa *et al*., 2015). Likewise, there are a number of endophytes that are dependable wellsprings of materials with pharmaceutical and/or agricultural possibilities as exemplified by taxol, subglutinol A and B, and peptide leucinostatin A (Stierle *et al*., 1993; Lee *et al*., 1995; Strobel and Hess, 1997; Nisa *et al*., 2015). It is significant to make reference to the point that the colonization of plant tissues by endophytes happens like those of plant pathogenic microbes and mycorrhizae (Lumyong *et al*., 2004; Nisa *et al*., 2015).

Most of the studies on vascular plant species revealed that there is the harboring of bacterial and/or fungal endophytes (Sturz *et al*., 2000; Tan and Zou, 2001; Arnold *et al*., 2000; Eljounaidi *et al*., 2016). It is then accordingly believed that bacterial endophytes stem from the communities' bacterial networks of the rhizosphere and phylloplane, just as from endophyte-infected seeds or plant materials. These endophytes access the plant by means of natural openings or wounds (Hallmann *et al*., 1997; Eljounaidi *et al*., 2016). Generally, the endophytic community is of dynamic structure and is affected by a few elements (see figure 2.10), which incorporates the plant development stage, plant physiological state, physicochemical state of the soil, and ecological conditions (Hallmann *et al*., 1997; Reiter *et al*., 2002; Ardanov *et al*., 2012; Mercado-Blanco and Lugtenberg, 2014; Eljounaidi *et al*., 2016).

Bacterial endophytes can decrease or forestall the pernicious impacts of certain pathogenic microbes and the advantageous results of bacterial endophytes on their host plant appear to emerge through different mechanisms (Ryan *et al*., 2008; Eljounaidi *et al*., 2016); mechanisms such as promotion of growth, antibiotic production (antibiosis), parasitism, initiating host defenses (induced systemic resistance, ISR), competition and interference of signal (quorum sensing) (Amer and Utkhede, 2000; Collins and Jacobsen, 2003; Jataraf *et al*., 2005; Jorjani *et al*., 2012; Mansoori *et al*., 2013; Eljounaidi *et al*., 2016). There are reports of numerous biocontrol bacterial endophytes displaying a mix of various mechanisms (Ongena *et al*., 2007; Eljounaidi *et al*., 2016).

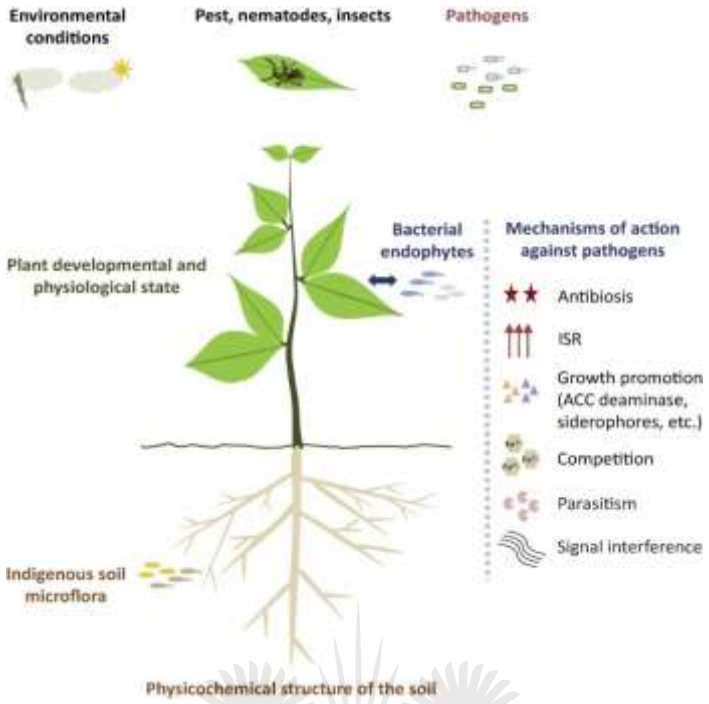


Figure 2.11: Schematic portrayal of the various elements impacting bacterial endophytes densities and dynamics: ecological conditions, the plant growth and physiological state, the indigenous soil microflora, and the physicochemical structure of the soil. In addition, showing the possible disease concealment mechanisms utilized by bacterial endophytes if there should be an occurrence of pathogenic attacks: antibiosis, competition, parasitism, ISR, development advancement and quorum sensing (Eljounaidi* et al.*, 2016).

Bacterial endophytes in one plant host are not limited to a single species however include a group of genera and species (Rosenblueth and Martínez-Romero, 2006; Eljounaidi *et al*., 2016). The most usually isolated bacterial genera are *Bacillus*, *Enterobacter*, *Pseudomonas* and Agrobacterium (Hallmann *et al*., 1997; Eljounaidi *et al*., 2016).

In spite of the fact that the initial discovery of endophytes goes back to 1904, this collection of microorganisms at first was neglected and did not truly get the necessary consideration until recently following the identification of paclitaxel (taxol) in the endophytic fungus *Taxomyces andreanae* that was isolated from *Taxus brevifolia*, the latter being the source of this significant drug employed in the treatment of cancer (Stierle *et al*., 1993; Stierle *et al*., 1995; Nisa *et al*., 2015). Until now, just a couple of plants have been widely examined for their endophytic biodiversity and their capability to synthesize bioactive secondary metabolites (Nisa *et al*., 2015).

2.7.2.1 Location of endophytes

A higher number of endophytes are said to be found in the roots of plants when compared to above-ground plant tissues (Rosenblueth and Martínez-Romero, 2004; Rosenblueth and Martínez-Romero, 2006; Eljounaidi *et al*., 2016). Since there are openings that already exist on the plant, the endophytes can also enter via the stomata or epidermis of the plant. Once within the plant, these microorganisms can disseminate to various parts of the plant or they may be localized at that point of entry. The microorganisms can colonize inter or intracellular spaces within the plant (Zinniel *et al*., 2002; Bernardi-Wenzel *et al*., 2010). According to Haque *et al*., (2015) endophytes utilize hydrolytic extracellular enzymes to penetrate the plant cells. In other cases, the endophytes are disseminated through seed transmission (Santoyo *et al*., 2016; Nchabeleng, 2017).

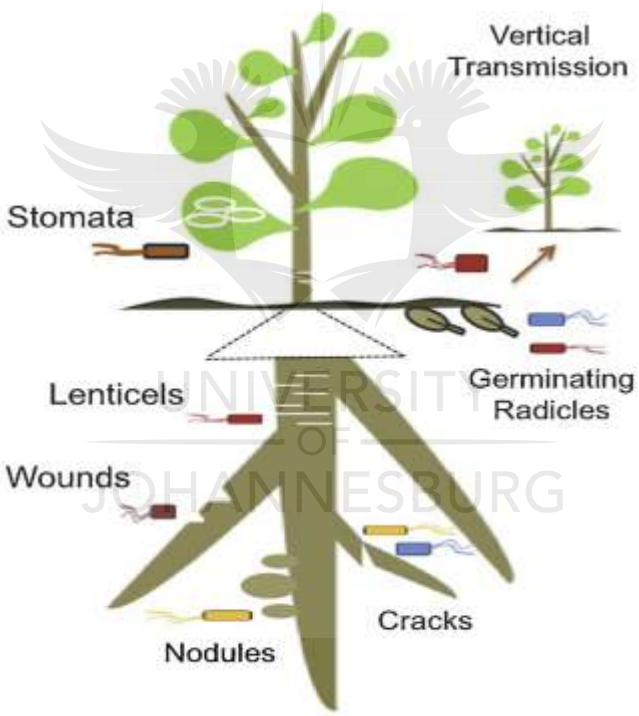


Fig. 2.12: Overview of endophytic bacterial method of passage into various plant tissues. The most widely recognized point of entry is through the roots, through primary and lateral root hair cells, root cracks and wounds, in addition to the hydrolysis of root cells. Other pointed of entry are stomata, especially on leaves and youthful stems; geminating radicles and lenticels. Rhizobia can colonize the inside plant tissues and form root nodules. Vertical seed transmission is additionally another approach to acquire endophytes through plant host generations (Santoyo et al., 2016).

2.7.2.2 Advantages of endophytes

Like the rhizosphere microorganisms, recent study revealed endophytes having beneficial impacts on their host plant thus suggesting them to be progressively productive in their association with their host than the rhizosphere organisms (Santoyo *et al*., 2016). Endophytes are found in many biological systems and are useful in diminishing biotic and abiotic stressors in plants by immune response stimulation, participating in antioxidant activities and phenylpropanoid metabolism and excluding plant pathogens by niche competition, whose initiation brings about plant defense, survival molecules and structural support (Ek-Ramos *et al*., 2019). As per metabolomic studies, endophyte genes identified with exact metabolites have been demonstrated to be effectively involved with plant growth promotion (PGP) via stimulating plant hormones production, for example, auxins and gibberellins or as plant defense agents against pathogenic microorganisms, insect pests and cancer but ecologically friendly and safe (Pandey *et al*., 2018; Ek-Ramos *et al*., 2019). Among plant microbiota, endophytic bacteria can be found in most plant species and be recouped from roots, stems, leaves, and some from flowers, fruits and seeds (Lodewyckx *et al*., 2002). These endophytic bacteria have the ability to synthesize various secondary metabolites with applications in farming, pharmaceutical, and biotechnological processes (Lodewyckx *et al*., 2002; Ryan *et al*., 2008; Strobel and Daisy, 2003; Ek-Ramos *et al*., 2019).

2.7.2.3 Endophytes as sources of natural products

Early investigations into various plant species uncovered the presence of one endophytic organism, at the minimum, with plants growing in distinct ecological settings to a great extent hosting novel endophytic microorganisms (Strobel, 2003; Ryan *et al*., 2008). Endophytes form a symbiotic relationship with their host plants, and it is believed that these microorganisms' have the capacity to act as biological defenses for the plants, protecting them against foreign phytopathogens. The protection system of these endophytes are applied directly, by the technique of releasing metabolites to attack any antagonists or destroy affected cells, and indirectly, by the technique of growth promotion or instigating host defense mechanisms (Alvin *et al*., 2014).

Anti-microbials or hydrolytic compounds are reported to be discharged by endophytes to stop the colonization of plants by pathogens, or keep insects and nematodes from infecting plants (Hallmann *et al*., 1998; Azevedo *et al*., 2000; Strobel, 2003; Berg and Hallmann, 2006). In different cases

through procedures known as “induced systemic resistance”, metabolites released by endophytes offered plants protection against other pathogenic microorganisms (Kloepper and Ryu, 2006). Similarly, in trying to outcompete cell apoptosis actuated by pathogenic microbes, endophytes are seen to help plant growth (Berg and Hallmann, 2006) and this plant growth advancement by endophytes are said to be exerted by mechanisms such as the creation of phytohormones (Tudzynski, 1997), nitrogen fixation, solubilization of minerals, for example, phosphorus (Richardson *et al*., 2009), synthesis of siderophores (O’Sullivan and O’Gara, 1992), or through enzymatic activities, like the suppression of ethylene by 1-aminocyclopropane-1-carboxylate deaminase (Glick *et al*., 1998). Endophytes provide their host plants with some advantages based on their in-born resistance to soil contaminants, their capacity to break down xenobiotics, or their activity as vectors to initiate degradative properties on plants, which significantly help in phytoremediation (Siciliano *et al*., 2001; Ryan *et al*., 2008).

Endophytes are known to produce comparable secondary metabolites as their host and a few of such compounds which are co-delivered by plants and their related endophytes are: camptothecin – an anticancer medication (Puri *et al*., 2005), podophyllotoxin - an anticancer medication lead compound (Puri *et al*., 2006), and azadirachtin - a natural insecticide (Kusari *et al*., 2012). There are various methods proposed for the concurrent production of these biological compounds. In a couple of cases, for example, that of gibberellin, the biosynthetic mechanism of the similar compound advances independently in plants and their microbial partners (Bömke and Tudzynski, 2009). On the other hand, horizontal gene transfer between the endophyte and its plant host has for some time been speculated, despite the fact that to this point this method has just been reported to happen between microbial endophytes (Taghavi *et al*., 2005). It has been unequivocally suggested, nonetheless, that collaborations among endophytes and their individual plant hosts add to the co-production of these bioactive compounds (Heinig *et al*., 2013).

There has been a developing enthusiasm on endophytes by virtue of their enormous ability to add to the discovery of novel biologically active compounds. It has been recommended that the direct biological relationship that exist between plants and their endophytes brings about the synthesis of a more prominent number and decent variety of bio-molecules when compared to epiphytes or soil-related microorganisms (Strobel, 2003). Also, the advantageous nature this relationship

recommends that endophytic bioactive molecules are probably going to have diminished toxic effects on cells, as these chemical compounds do not cause harm to the eukaryotic host framework. This is notable to the medical communities and networks as potential medications may not have severe adverse effects on human cells (Alvin *et al*., 2014).

One of the best accounts of endophytic natural products is the multibillion-dollar anticancer drug "Taxol" (paclitaxel) which was at first isolated from the Pacific yew tree, *Taxus brevifolia*, a customary therapeutic plant utilized by Native Americans (Gunther, 1945; Wani *et al*., 1971; Alvin *et al*., 2014). Examinations of endophytes from this plant uncovered that a couple of fungi, like, *Taxomyces andreanae*, likewise produced comparable compound (Stierle *et al*., 1995). The organic creation of this compound in Taxus plants have been characterized. While horizontal gene transfer has for some time been proposed for the biosynthesis of Taxol in endophytes, it has as of late been demonstrated that the endophyte did not contain any genome sequences with significant homology to the Taxol biosynthetic gene from *Taxus* spp. (Heinig *et al*., 2013). This suggests that Taxol biosynthesis gene in the endophyte may have developed independent of its host plant. However, this example backs-up the justification that plants employed in traditional medicine practices can be utilized as the starting point to explore endophytes for their production of bioactive compounds (Alvin *et al*., 2014).

In all the natural sources employed in drug discover studies, the capability of endophytes have been reported in the last decade (Pirttilä and Frank, 2011) and are still being explored and reported. The increased number of review articles, original research articles and patents being published indicates that studies on the isolation and identification of metabolites from microorganisms, particularly endophytic bacteria and fungi, are rapidly expanding (Tejesvi *et al*., 2011). The presence of numerous metabolites of fungal origin in the pharmaceutical market indicates the capability of microorganisms as a significant source of lead drugs, for instance, semisynthetic or synthetic penicillins and cephalosporins, the antibiotic polyketide griseofulvin (Likuden M), the antibacterial terpenoid fusidic acid (Fucidin), macrolides, chloramphenicol, statins, and the ergot alkaloids, for example, ergotamine (Ergo-Kranit) (Butler, 2008; Hamilton-Miller, 2008; Parry *et al*., 2011). Table 2.4 provides a good summary of the different compounds or drugs that have been developed from endophytic bacteria.

2.7.2.4 Endophytic bacteria products as antimicrobials

The most commonly isolated endophytes are the fungal endophytes. Bacterial species associated with plants are quite limited and the most common genera is *Pseudomonas*. *Pseudomonas* species are epiphytic, pathogenic and endophytic and have been reported across every continent (Lodewyckx *et al*., 2002). Some species of this genera produce compounds that are phytotoxic as well as antibiotics. On the other hand, *Streptomyces* sp. strain NRRL 30562 from *Kennedia nigriscans* plant produces a broad-spectrum antibiotics called munumbicins (Castillo *et al*., 2002). These antibiotics possess various biological activities according to the organism being targeted. However, munumbicins demonstrates antimycobacterial activity against multidrug-resistant *Mycobacterium tuberculosis* as well as other drug-resistant bacteria and against some Gram-positive bacteria (e.g. *Bacillus anthracis*) (Ek-Ramos *et al*., 2019).

The first example of plants as reservoirs of actinomycetes (the world's basic source of antibiotics), is seen in the isolation of a streptomycete endophyte: *Streptomyces* sp. strain RRL 30562. Previously, practically all actinomycetes utilized in the production of modern antibiotic were isolated from soil. But, these days, over 30 of those actinomycetes are reported as endophytes and hold antibiotic properties. In addition, actinomycetes endophytes are now being studied and considered for use in plant disease control (Gutierrez *et al*., 2012). Table 2.4 shows examples of bioactive compounds from endophytic bacteria.

2.7.2.5 Endophytic bacteria products as anticancer agents

Cancer remains the leading cause of death all over the world despite different therapeutical advances aimed at controlling this disease (Tan and Zou, 2001; Chen *et al*., 2013; Ek-Ramos *et al*., 2019). Drug resistance and the series of side effects associated with conventional chemotherapy and radiotherapy are some of the major challenges in the treatment of cancer. Thus, the search for new, highly effective antitumor agents with lesser or no detrimental effects (Ek-Ramos *et al*., 2019).

Compounds isolated from actinomycete endophytes have been reported useful as antimicrobial agents as well as cytotoxic agents against tumor cells and members of this Gram-positive group of bacteria are endophytes associated with numerous plant species (Eljounaidi *et al*., 2016). Extracts from endophytes are preferred to chemotherapy agents based on their limited side effects and

antitumor activity efficiency, in addition to their lesser toxicity to normal cells and high activity against microorganisms that are drug resistant. Therefore, endophytic natural secondary metabolites have in recent times attracted attention as possible anticancer chemotherapeutic and cancer-chemopreventive drugs (Filho, 2018). Natural secondary metabolites from gram positive bacterial endophytes have shown to be one of the most definitive sources of alternative treatment (Gutierrez *et al*., 2012; Chen *et al*., 2013) including antitumor agents such as coumarins, enediynes, flavonoids, naphthoquinones, polysaccharides, anthracyclines, anthraquinones, etc. (Igarashi *et al*., 2007; Taechowisan *et al*., 2007; Chen *et al*., 2013; Filho, 2018).

Since the initial reports on the prospective use of endophytic secondary metabolites in industrial processes, more evidence have come up, indicating that Gram-positive bacteria are among the most prominent sources of novel compounds with likely use in the medical, pharmaceutical and agricultural sector owing to their plant growth promoting, antimicrobial and anticancer activities (Villarreal-Delgado *et al*., 2018). Natural products have remained consistent as sources of drug leads. Between the 1940s and 2006, 42% of the 175 anticancer drugs approved for use were either natural products or compounds derived from natural products (Olano *et al*., 2009). A good number of antitumor drugs are natural products isolated from microorganisms and/or plants growing in environmental niches (Ek-Ramos *et al*., 2019).

Table 2.4: Examples of various bioactive natural products from bacterial endophytes.

Endophytic bacteria	Compound/drug name	Plant sources	Bioactivity	References
Streptosporangium oxazolinicum K07-0450T	Spoxazomicins A-C	Orchids	Antitrypanosomal	(Inahashi *et al*., 2011; Inahashi *et al*., 2011; Brader *et al*., 2014)
Serratia marcescens,	Oocydin A	*Rhyncholacis penicillata*	Antifungal	(Strobel *et al*., 2004)
Pseudomonas viridiflava	Ecomycins	Grass species	Antifungal lipopeptides	(Miller *et al*., 1998)
P. viridiflava strain EB274 and EB227	Ecomycins B and C	--	Antifungal lipopeptides	(Harrison *et al*., 2009)
Streptomyces sp. MSU-2110	Coronamycin	*Monstera sp.*	Bioactivity against *Plasmodium falciparum* – antimalarial.	(Ezra *et al*., 2004; Martinez-Klimova *et al*., 2017)
Streptomyces sp. strain GT2002/1503	Xiamycin-A	*Bruguiera gymnorrhiza*	Anti-HIV activity	(Ding *et al*., 2010)
Streptomyces aureofaciens	5,7-dimethoxy-4-pmethoxylphenylcoumarin. 5,7 dimethoxy-4-phenylcoumarin.	*Zingiber officinale*	Antifungal	(Taechowisan *et al*., 2005; Martinez-Klimova *et al*., 2017)
Colletotrichum dematium	Collutellin A	*Pteromischum* spp	Antifungal	(Ren *et al*., 2008; Martinez-Klimova *et al*., 2017)
Paenibacillus polymyxa	Fusaricidin A–D	Wheat, Lodge pine, Green beans, *Arabidopsis thaliana* and Canola	Antifungal	(Beck *et al*., 2003; Li *et al*., 2007; Pandey *et al*., 2017)
Streptomyces NRRL 30562	Munumbicins A, B, C and D	*Kennedia nigriscans* (Snake vine)	Antimicrobial, antifungal and antimalarial.	(Castillo *et al*., 2002; Castillo *et al*., 2006)

Endophytic bacteria	Compound/drug name	Plant sources	Bioactivity	References
Streptomyces NRRL30566	Kakadumycin A	*Grevillea pteridifolia* (Grevillea tree)	Antibiotics	(Castillo *et al*., 2003)
Paenibacillus sp. IIRAC-30	C15- lipopeptide	*Manihotes culenta* (Cassava)	Antifungal	(Canova *et al*., 2010)
Bacillus licheniformis and *Bacillus pumilus*		*Platycodon grandiflorum* (Balloon flower)	Antifungal compound	(Asraful Islam *et al*., 2010)
Bacillus mojavensis	Leu7 – surfactin	-	Antifungal	(Snook *et al*., 2009)
Shewanella sp. and *Pseudomonas* sp.	2-amino-3- quinolinecarbonitrile and boric acid	*Ageratum conyzoides*	Antibacterial	(Fitriani *et al*., 2015)
Bacillus amyloliquefaciens	Fengycin homologues and surfactin homologues	*Scutellaria baicalensis* Georgi	Antimicrobial	(Sun *et al*., 2006)

2.8 CONCLUSION

Throughout the years, natural products from plants have been identified as a source of curative agents which have assumed a fundamental role in the identification of new chemical elements for drug discovery. There is a developing upsurge in call for traditional and herbal solutions for relieving different illnesses among various communities all through the world. Comprehensive screening of medicinal plants is required for the identification, isolation and development of new bioactive molecules that may help in decreasing human health problems (Kumar *et al*., 2015). While plants are good wellsprings of bioactive compounds, the effects of constant harvesting of plants on our environment cannot be overlooked and endophytes should be considered since its isolation requires only a few parts of the plant and they also produce similar phytochemical compounds as their host plant(s).

According to Tan and Zou, (2001), the rationale behind some endophytes producing phytochemicals that are distinctive to their host plant could be traced to a genetic recombination of the endophytes and the host plants which arose from evolutionary time. Since endophytes are able to synthesize similar rare and important bioactive secondary metabolites are their host plant, the need to constantly harvest endangered and slow-growing plants would be greatly reduced and in turn the diminishing biodiversity of the world could be preserved. Additionally, a microbial source of a product of high value could be easier, more economical and more effectively produced resulting in reduction in market price (Gutierrez *et al*., 2012). Hence, more detailed information on plant species and the biology of their microbial community would be remarkably helpful in leading the search for bioactive secondary metabolites (Li *et al*., 1996).

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CHAPTER THREE

ISOLATION AND CHARACTERIZATION OF BACTERIAL ENDOPHYTES FROM *Solanum mauritianum* Scop.

3.0 ABSTRACT

Plants house a diverse assembly of microbial communities inside their bodies that are called endophytes. Endophytes are microorganisms living in the internal tissues of plants without causing any overt symptoms. The bacterial endophytes present in aerial parts of *S. mauritianum* harvested around the Doornfontein area of Johannesburg were isolated from surface sterilized plant parts and were identified based on their morphological and microscopic characteristics and on their molecular characteristics using 16S rRNA sequencing. The results showed that a total of 7 bacterial endophytes were isolated including species of the *Pantoea, Xanthomonas*, *Arthrobacter* and *Bacillus* genera. This study is the first of its kind on *S. mauritianum*. Since its been reported that both plants and their associated microorganisms are able to produce various bioactive compounds that can be medically important, it becomes necessary for more studies to be carried out on these two aspects to provide more information and establish a foundation of the host-endophyte interaction of *Solanum mauritianum* and the consequent secondary metabolites they both produce including their potential applications.

Keywords: Bacterial, endophytes, *Solanum mauritianum*, 16S rRNA.

Results from this chapter have been published in the *International Journal of Environmental Research and Public Health*.

3.1 INTRODUCTION

Assorted microbial communities made up of bacterial, archaeal, protistic and fungal taxa inhabit the internal parts of all plants (Hallmann *et al*., 1997; Hardoim *et al*., 2015; *Tian et al*., 2017). These microorganisms with their endophytic ways of life assume significant roles in plant development, diversification, growth and wellness. Insight into the complexity of plant microbiome is being investigated with increasing information and awareness on endophytes (Turner *et al*., 2013; Hardoim *et al*., 2015; Tian *et al*., 2017). Plant-endophyte interactions could either be characterized as pathogenic or mutualistic. This characterization could be founded on various biotic and abiotic factors which may include: the genotype of plants and microorganisms, ecological conditions and the dynamic system of connections inside the plant biome (Hardoim *et al*., 2015).

Previously, “endophyte” as a term was commonly associated with fungal species living within plants, but researchers have since discovered that bacterial species also colonize internal parts of plants (Chanway, 1996; Hallmann *et al*., 1997; Hardoim *et al*., 2015). Plants do not exist as sole entities, they partner intimately with the microorganisms present in their environment and with those living inside them. The approach of the idea of "plant microbiome", (that is, the aggregate genomes of microorganisms living with plants) has made inventive thoughts on plant evolution where specific forces act not simply on the plant genome itself but on the entire plant, which also includes the microbial community associated with the plant (Rosenberg *et al*., 2009; Hardoim *et al*., 2015).

Endophytes are commonly defined as “those bacteria that can be isolated from surface-disinfested plant tissue or extracted from within the plant, and that do not visibly harm the plant.” This is the experimental definition given by (Hallmann *et al*., 1997). In the course of recent decades, this definition has been substantial for cultivable species in many laboratories around the globe. Nevertheless, this definition appears to be less appropriate for non-cultured species upon the establishment of molecular detection techniques now applied in endophyte

research owing to the suspected deficiency to adequately eliminate nucleic acids after surface disinfection of plant (Garbeva *et al*., 2001; Hardoim *et al*., 2015).

The occurrence of beneficial microbes within plants was additionally affirmed by research done in the late nineteenth century and the mid twentieth century (Laurent, 1889; Hardoim *et al*., 2015). Notwithstanding, various ideas on the presence of plant-beneficial endophytes prevailed around then (Laurent, 1889; Schneider, 1894; Smith, 1911; Hardoim *et al*., 2015). At the present time, it has been established that plants fill in as hosts for different microbial endophytes including fungi, bacteria, archaea, algae (Trémouillaux-Guiller *et al*., 2002) and amoebae (Müller and Döring, 2009).

Higher plants have interactions with various groups of bacteria and fungi. There has been reports of genetic connections between the relationship of plants with Arbuscular Mycorrhizal Fungi (AMF) and root nodule symbiosis (Kanamori *et al*., 2006; Markmann *et al*., 2008), which proposes that portions of endophytic bacterial and fungal populations have coevolved with one another just as with their host plant (Hardoim *et al*., 2015).

Endophytic bacteria are found in the inner parts of plants and do not cause any disease symptoms to the plant. There has been reports indicating that bacterial endophytes exist in diverse tissue types across a wide range of plants, which suggests an that they ubiquitously exist in almost all higher plants (Hallmann *et al*., 1997). After infiltration, endophytes may inhabit the plant systemically with biofilms and colonies of bacteria latently living in the intercellular spaces, inside the plant cells and the vascular tissues (Jacobs *et al*., 1985; Hurek *et al*., 1994; Di Fiore and Del Gallo, 1995). While the relationship between plants and their resident endophytic bacteria is yet to be entirely comprehended, numerous endophytic isolates have indicated advantageous effects on their host plants and may assume a significant role in plant physiology (Ulrich *et al*., 2008).

With increasing cases of life-threatening infections coupled with drug-resistant microbes, attention has been drawn towards discovery of compounds with novel pharmacological activities. Therefore, bioprospecting of secondary metabolites produced by endophytes from medicinal plants offers a vast potential lead to the discovery of compounds with novel pharmacological activities (Alvin *et al*., 2014). The store of microbes (especially endophytic bacteria) hidden within host plants are poorly investigated and are underexplored as potential sources of novel secondary metabolites for medical and commercial purposes. Seeing as there are so many bacterial endophytes found in numerous unique biological niches (higher plants) that grow in various environments, interesting possibilities exist in the venture into the wild and unexplored territories of the world of natural products and engaging the discovery of endophytes, their biology and potential usefulness (Mehanni and Safwat, 2010). Microbe-plant interactions are far from being fully understood. Nevertheless, more evidence shows plant-associated microorganisms provide substantial benefits to agriculture, industry, and the environment.

This study is aimed at isolating and identifying the possible bacterial endophytes present in *Solanum mauritianum* which is a plant used in South African traditional medicine. These endophytes will be further explored for some of their beneficial metabolic products.

3.2 MATERIALS AND METHODS

3.2.1 Sample collection

Fresh, healthy (showing no apparent symptom of disease) aerial parts (leaves, stems, ripe and unripe fruits) of *S. mauritianum* plant were collected from the University of Johannesburg, Doornfontein Campus, located in the Doornfontein area of Johannesburg, South Africa. The samples were transported to the laboratory where the plant was thoroughly washed with sterile distilled water and used within four hours.

3.2.2 Endophytes isolation and Identification

The plant parts were surface sterilized separately using the method described by Jasim *et al*. (2014) and Nchabeleng, (2017). Briefly, each sample (approximately 10 g) was treated with 5 % Tween 20 (enough to cover the plant material) and shaken vigorously for five minutes. The Tween 20 was removed by rinsing several times with sterile distilled water. The sample was then disinfected with 70 % ethanol for one minute. Traces of the ethanol were removed by rinsing with sterile distilled water five times. The sample was then treated with 1 % Sodium Hypochlorite (NaHClO) for ten minutes and rinsed five times with sterile distilled water. The last rinse was used as a control and 100 µL of it was plated on Nutrient Agar (NA) The sample was then macerated in sterilized phosphate buffered saline (PBS) with the outer surface trimmed out. The macerated sample was serially diluted up to 10^{-3} dilution and each dilution inoculated (using spread plate method) in triplicates on and Nutrient Agar (NA) (for bacteria enumeration). The NA plates were incubated at 30 °C, (IncoTherm, Labotec, South Africa) for 5 days. Effectiveness of the sterilization was monitored on the wash control plate, with growth indicating poor sterilization. Under such circumstances, the plates were discarded, and the sterilization repeated.

Distinct colonies were selected and sub-cultured on the appropriate NA to obtain pure isolates. Pure bacterial isolates were preserved in 50 % glycerol on a ratio of 1 mL glycerol: 1 mL overnight broth culture and stored at -80 °C.

3.2.3 Morphological identification of bacterial endophytes

Gram stain technique as described by Collins *et al*. (2004) was done to determine microscopic cell morphology. Gram stain slides were observed with a compound bright-field microscope (OLYMPUS CH20BIMF200) with 1000× magnification.

3.2.4 Molecular identification

3.2.4.1 Genomic DNA extraction, Polymerase chain reaction and sequencing

Pure colonies of each bacterial endophyte isolate were obtained from nutrient agar plates and inoculated into nutrient broth and grown overnight at 30 °C. Cultures were centrifuged at 13000 × g for 5 min and supernatants discarded. The DNA was extracted using Bacterial DNA kit (Zymo Research, catalog NO R2014) according to the manufacturer's instructions. The extracted DNA was quantified using the NanoDrop ND-2000 UV-Vis spectrophotometer (Thermo Fisher scientific, USA). The 16S rRNA gene of each bacterial endophyte was amplified following protocol described by Tsuchida *et al*., (2002). Briefly, the 16S rRNA gene was amplified using the primers (16S-27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 16S-1492R: 5'-CGGTTACCTTGTTACGACTT-3') with 2x Polymerase chain reaction (PCR) master mix with standard buffer under a temperature profile of 95 °C for 4 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. The PCR products were cleaned with ExoSAP-it™ following manufactures' instructions and sequencing was performed at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

3.2.4.2 Phylogenetic analysis

The obtained sequences were screened for chimeras using DECIPHER23 (Wright *et al*., 2012). The 16S rRNA gene sequences were subjected to BLAST (v.2.6.0) analysis at National Center for Biotechnology Information (NCBI) against rRNA sequence database of bacteria and archaea to identify closest bacterial species. Bacterial species with 97-100% similarities were selected for phylogenetic analysis. Alignments of nucleotides sequences (isolate and species obtained through BLAST) were performed using MUSCLE with default options. Phylogenetic trees were constructed using a Neighbor Joining (NJ) method based on the Tamura-Nei model Tamura *et al*., (2013). A total of 1000 replications were used for bootstrap test. All branches with greater than 50% bootstraps were seen as significant (Soltis and Soltis, 2003). All positions with gaps and missing nucleotide data were eliminated. All evolutionary analyses were conducted in MEGA 7.27. The 16S rRNA gene sequences of

bacterial isolates identified in the study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with the accession numbers as stated in Table 1. The assigned names of the bacterial isolates were based on the BLAST homology percentages as well as phylogenetic results.

3.3 RESULTS AND DISCUSSIONS

In this study, a total of seven bacterial endophytes were isolated from the different parts of *S. mauritianum* and the accession numbers of the isolated endophytes are shown in Table 3.1. All isolates had between 97 – 100 % similarities with other strains obtained from GeneBank. The results of the effectiveness of the surface sterilization method showed no microbial growth in the control plates, indicating that the isolates are endophytes. Relationships between the isolated bacterial endophytes and other species of the same families are shown in Figures 3.1 and 3.2. The phylogenetic analysis showed that the endophytic bacterial isolates aligned with various closely related bacterial species.

Table 3.1: Identification of different endophytes from *S. mauritianum* parts.

Bacterial isolate	Accession number	Closest relatives in NCBI	Identity (%)	Tissue	Phylum; Class; Order	Classification	Macroscopic identification	Microscopic identification
NU 01	MK070326	*Pantoea ananatis* strain P5 (FJ796221)	99	Stem	Proteobacteria; Gammaproteobacteria; Enterobacteriales	*Pantoea ananatis*	Round, white, raised and entire colonies	Gram negative rods
NU 02	MK070327	*Luteibacter* sp. strain BSNB-0721 (MK643286)	98.95	Ripe fruit	Proteobacteria; Gammaproteobacteria; Xanthomonadales	*Xanthomonas* sp.	Irregular, yellow, raised and entire colonies	Gram negative rods
NU 03	MK070328	*Pantoea eucalypti* strain EV2 (FM202484)	99	Stem	Proteobacteria; Gammaproteobacteria; Enterobacteriales	*Pantoea eucalypti*	Round, Creamy, irregular and raised colonies	Gram negative rods
NU 04	MK070329	*Bacillus safensis* strain Rb1S1 (MK342521)	100	Leaves	Firmicutes; Bacilli; Bacillales	*Bacillus safensis*	Round, creamy, flat and entire colonies	Gram positive rods
NU 05	MK070330	*Pantoea vagans* strain UFLA WFC767 (KY660463)	99	Stem	Proteobacteria; Gammaproteobacteria; Enterobacteriales	Pantoea vagans	Round, translucent, convex and entire colonies	Gram negative rods
NU 06	MK070331	*Bacillus licheniformis* strain V46 (MK229112)	99	Green fruit	Firmicutes; Bacilli; Bacillales	*Bacillus licheniformis*	Round, creamy, flat and entire colonies	Gram positive rods
NU 07	MK554845	*Arthrobacter* sp. Gr-41sp (KF010645)	97.59	Leaves	Actinobacteria; Actinobacteria; Actinomycetales	*Arthrobacter* sp.	Filamentous, white, raised and undulate colonies	Gram positive rods

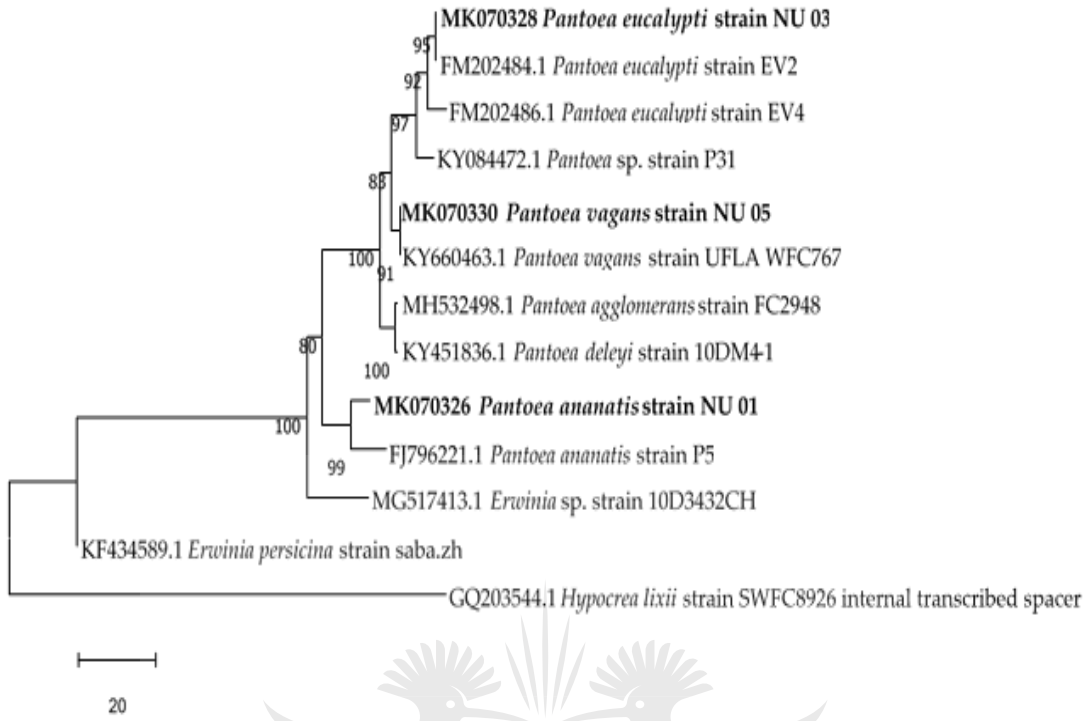

Figure 3.1: Neighbour joining tree based on 16S rRNA gene sequence of endophytic bacteria isolated from the stem of S. mauritianum and other similar species selected from GeneBank.

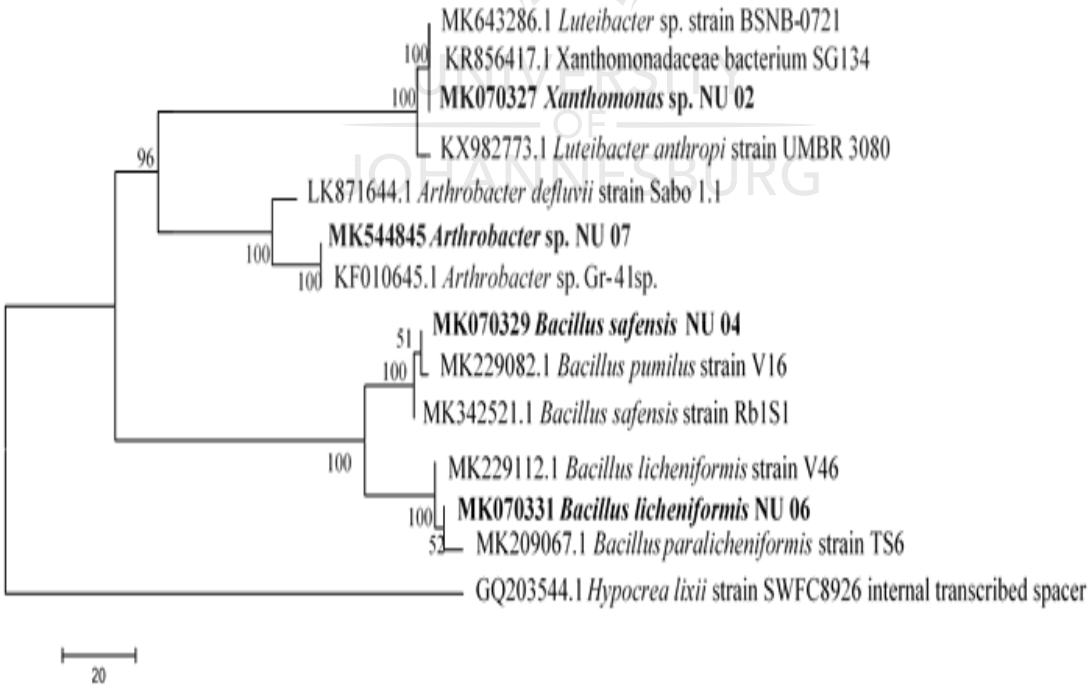

Figure 3.2: Neighbour joining tree based on 16S rRNA gene sequence of endophytic bacteria isolated from the ripe and unripe fruits and leaves of S. mauritianum and other similar species selected from GeneBank.

Plants are inhabited by diverse microbial communities, ranging from the rhizosphere and phyllosphere (Compant *et al*., 2010). These microorganisms maintain contact with their host plants and play vital roles in plant development, growth, and fitness, as well as decontaminating polluted soils (Hardoim *et al*., 2015; Tian *et al*., 2017). Endophytes engage in these intimate interactions with their host plants without inflicting infections or other negative effects, resulting in mutualistic relationships in most cases (Hardoim *et al*., 2015).

Endophytes are known to vary in diversity based on seasonal collection or sampling time, plant age, plant tissue type and environment (Jasim *et al*., 2014). Recent studies have equally documented *Pseudomonas, Bacillus, Pantoea* and *Enterobacter* as bacterial endophytes in plants (Duijff *et al*., 1997; Hallmann *et al*., 1997; Akinsanya *et al*., 2015). The assignment of bacterial isolates to a given species require further phenotypic and molecular characterization.

Based on this study, the *Pantoea* species isolates – NU 01, NU 03 and NU 05 had 99% similarities to *Pantoea vagans* strain UFLA WCF767, *Pantoea agglomerans* strain FC2948, *Pantoea ananatis* strain P5 and *Pantoea eucalypti* strains EV2 and EV4. There are reports showing the association of *Pantoea* species with plants (Ferreira *et al*., 2008). *Pantoea agglomerans* have been shown to act as a biocontrol agent on cotton (Medrano and Bell, 2007), produce antibiotics that inhibit *Erwinia amylovora* (Wright *et al*., 2001) and promote plant growth (Procópio, 2004; Feng *et al*., 2006). The isolate *Xanthomonas* isolate – NU 02 had 98.95 % similarity to *Luteibacter* sp. strain BSNB-0721; both of which are from phylum: Proteobacteria, order: Xanthomonadales and family: Rhodanobacteraceae indicating that they are both closely related. *Bacillus* isolates - NU 04 and NU 06 had 100 % similarity to *Bacillus safensis* strain Rb1S1 and 99 % similarity to *Bacillus licheniformis* strain V46 respectively. The *Arthrobacter* isolate – NU 07 had 97.59 % similarity to *Arthrobacter* sp. Gr-41sp. *Pantoea* spp., *Bacillus* spp. *Arthrobacter* spp. and *Xanthomonas* spp. are all common soil bacteria which can get into from through the plant roots. These bacterial species have also been previously isolated from maize, rice and medicinal plants as bacterial endophytes (Egamberdieva *et al*., 2017; Walitang *et al*., 2017).

Amongst plant microbiota, bacterial endophytes can be identified and isolated from the roots, leaves, stems, and a few from flowers, fruits, and seeds of most plant species (Lodewyckx *et al*.,

2002; Mercado-Blanco and Lugtenberg, 2014) and biotechnological explorations of these bacterial endophytes have revealed their potential to produce a variety of secondary metabolites with application in agriculture, pharmaceutical and industrial biotechnology (Lodewyckx *et al*., 2002; Strobel and Daisy, 2003; Ryan *et al*., 2008). It has been reported that the most abundant metabolite producing bacterial endophytes found in various environments are Gram-positive *Bacillus* and *Streptomyces* species (Reinhold-Hurek and Hurek, 2011; Frank *et al*., 2017; Ek-Ramos *et al*., 2019).

These endophytes isolated from *S. mauritianum* will be further explored to understand their biological activities and the type of metabolites they produce.

3.4 CONCLUSION

This study gives a report on the bacterial endophytes of different species present in the aerial parts of *S. mauritianum* and supports our second hypothesis of *S. mauritianum* being host to diverse endophytic bacteria. It validates other reports about the different parts of one plant harboring diverse species of bacterial endophytes. These endophytes will be further explored to identify the secondary metabolites they produce and the biological usefulness of these metabolites. To the best of our knowledge, this is the first report on the bacterial endophytes of *S. mauritianum*.

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CHAPTER FOUR

SCREENING OF CRUDE SECONDARY METABOLITE EXTRACTS FROM *Solanum mauritianum* Scop. AND ITS BACTERIAL ENDOPHYTES FOR ANTIBACTERIAL ACTIVITY, CYTOTOXICITY AND ANTICANCER ACTIVITY.

4.0 ABSTRACT

Crude extracts from plants and endophytes are very useful in traditional medicine and drug discovery research. These crude extracts have been proven to be active against disease symptoms and cancers due to the presence of various bioactive secondary metabolites. The aim of this study was to check the bioactive potential of crude secondary metabolites from *Solanum mauritianum* Scop. and its bacterial endophytes. Extracted crude secondary metabolites were screened for antibacterial activity against 11 pathogenic bacteria and two human cancer cell lines using the MIC and MTS assays respectively. RTCA assay was carried out on the most active crude extract. The result showed antibacterial activity against the test pathogenic bacteria with MIC ranging from 0.031 to 16.00 mg/mL across all the tested crude extracts. For the endophytes, *Arthrobacter* sp. crude extract was the most active with MIC ranging from 0.063 to 1.000 mg/mL across all 11 pathogens while *Xanthomonas* sp. crude extract was the least active with MIC of 0.500 – 16.00 mg/mL. For the plant parts, the unripe fruits has the most noteworthy activity with MIC of 0.031 – 1.000 mg/mL while the leaf extract was the least active with MIC range of 0.125 to 4.000 mg/mL. An increase in cell proliferation was observed when A549 Lung carcinoma and UMG87 glioblastoma cell lines were treated with treated with both the plant and endophytes' crude extracts. The most active crude extract against glioblastoma cell line was *S. mauritianum* ripe fruit coat which reduced cell viability by 37.9417 % and the RTCA assay showed it had a concentration dependent activity. Statistical analysis of the samples' effected on cell viability based on concentration was found to be non-significant as $P \geq 0.05$. The antibacterial activity results from this study shows that this plant and its bacterial endophytes can be explored to extract and produce antimicrobial compounds.

Keywords: Antibacterial, anticancer, crude extracts, endophytes, *S. mauritianum*

Results from this chapter have been published in the *International Journal of Environmental Research and Public Health*.

4.1 INTRODUCTION

In the control of malignancies and infectious diseases, plant derived extracts or other natural products (like microorganisms) are of significant value owing to their low cytotoxicity and drug resistance (Mbaveng *et al*., 2011). Typical examples are taxol, combretastatins, camptothecin used in cancer treatment (Srivastava *et al*., 2005) and vincristine/vinblastine, morphine, quinine used in the treatment of various diseases (Licciardi and Underwood, 2011) are all plant derived products. Modification of natural products or the specific isolation of compounds from medicinal plants are the basis for the research and discovery of new drugs (Srivastava *et al*., 2005). Nevertheless, while medicinal plants are rich sources of drugs, they are being over exploited due to over-harvesting which leads to the destruction of ecological habitat. Therefore, there is the need to preserve endangered medicinal plants and develop new alternative sources for harvesting novel bioactive compounds (Chen *et al*., 2014). In view of this need to preserve the biodiversity, endophytes which are microorganisms that are in symbiotic relationship with plants are considered good sources of new bioactive compounds.

In a single plant or microbe, endophytes are able to produce numerous bioactive metabolites which are good drug sources for the treatment of diseases and are applied in the medicine, food, agriculture and cosmetic industries (Strobel and Daisy, 2003; Jalgaonwala *et al*., 2011; Shukla *et al*., 2014). Endophytes are sources of a wide range of bioactive secondary metabolites with exceptional structures which include groups of compounds like alkaloids, flavonoids, phenolic acids, quinones, steroids, tannins, terpenoids, etc. (Tan and Zou, 2001; Pimentel *et al*., 2011). These bioactive metabolites being used as antibiotics, antiparasitics, immunosuppressants, antioxidants, anticancer agents and agrochemicals (Gunatilaka, 2006). Endophytes have been identified as potential useful sources of bioactive secondary metabolites most useful in anticancer applications (Schulz *et al*., 2002; Strobel *et al*., 2004; Kharwar *et al*., 2011; Ambrose *et al*., 2013; Chen *et al*., 2014).

While natural bioactive secondary metabolites synthesized by endophytic microbes have shown prospects in human health concerns, the drug industry still makes use of synthetic products because of economic reasons (Strobel *et al*., 2004; Ambrose *et al*., 2013). In the last 10 – 15 years, there has been an increase in cases antibiotic-resistant fungi and bacteria which cause human infections (Norrby *et al*., 2005; Carvalho *et al*., 2012) and due to this, recent drug discovery programs are

exploring new sources of antibiotics with novel mechanisms of action (Butler and Buss, 2006; Carvalho *et al*., 2012).

Additional aspect of such drug discovery programs include the search for new drugs to treat cancer. Cancer has been a health problem with great medical, economic and social effects around the world. A number of existing cancer drugs are cytotoxic to malignant cells as well as normal cells (Anazetti *et al*., 2003) and a major step in developing less toxic anticancer drugs is in the search for new sources of selectively cytotoxic compounds (Carvalho *et al*., 2012).

According to Newman and Cragg, (2007), the most consistent and productive source for first-in-line drugs are natural products derived from medicinal plants and microorganisms. Lately, the interest in the discovery of exceptional pharmacological agents from endophytes has greatly increased (Strobel and Daisy, 2003; Deshmukh *et al*., 2009). To promote the fight against resistant microbes and cancer, plant and microbial natural products play important roles as sources of compounds with therapeutic properties, which accounts for about 75 % drugs used clinically (Kornienko *et al*., 2015; Yuan *et al*., 2016).

This study was aimed at extracting the crude secondary metabolites from aerial parts of *Solanum mauritianum* and their isolated bacterial endophytes and testing these crude extracts for bioactivity against common bacterial pathogens and human cancer cells.

4.2 MATERIALS AND METHODS

4.2.1 Extraction of Crude Secondary Metabolites from endophytes

The seven isolated and identified bacterial endophytes (see Chapter 3) were labelled as follows: *P. ananatis* = NU 01 Pa; *Xanthomonas* sp. = NU 02 Xs; *P. eucalypti* = NU 03 Pe; *B. safensis* = NU 04 Bs; *P. vagans* = NU 05 Pv; *B. licheniformis* = NU 06 Bl; *Arthrobacter* sp. = NU 07As.

Secondary metabolites were extracted from the endophytic bacteria using the method described earlier (Maloney *et al.*, 2010) with modifications. The endophytic bacteria isolated from S. mauritianum were cultured in three 5 L Schott bottles each containing 3 L of a nutrient broth and shaken at 200 rpm at 27 °C for seven days. After seven days of cultivation, sterilized Amberlite®

XAD7HP 20-60 mesh resin (60 g/L) (Sigma-Aldrich, South Africa BCBR6696V) was added to adsorb the organic products, and the culture and resin were shaken at 200 rcf for 2 h. The resin was filtered through cheesecloth and washed three times with 250mL of acetone for each wash. The acetone soluble fraction was concentrated using a rotary evaporator and a dark brown viscous extract was obtained.

The extract was transferred into a measuring cylinder and based on the volume; ethyl acetate was added in a 1:1 ratio (v/v). The mixture was shaken vigorously for 5-10 minutes, poured into a separating funnel and allowed to separate; this was done until the dark brown viscous liquid obtained after removing the acetone became a light-yellow liquid. The ethyl acetate fraction was removed using a rotary evaporator and the extract was stored in an amber bottle in a cool dry place until analysis was done. The light-yellow liquid was evaporated (to ensure there was no extract lost) leaving behind no reasonable extract, no further analysis was done on this.

4.2.2 Extraction of *Solanum mauritianum* plant parts

The methods described by Fomogne-Fodjo *et al*. (2014) and Uche-Okereafor, (2016) were used for the preparation of the crude extracts of *S. mauritianum*. The dried plant part was blended into a fine powder with a shop-bought coffee mill and 200 g of plant powder was weighed into a Schott bottle, 2000 mL of a methanol/chloroform (50:50, v/v) solution was added and the Schott bottle left on a platform shaker for three days. The extract suspension was filtered through Whatman No. 1 filter paper and evaporated to dry out the solvent using a rotatory evaporator with consideration to the boiling points of the extracting solvents. The process was repeated another two times to ensure maximal extraction of compounds. The crude extract was collected in a beaker and placed in a desiccator to dry out completely.

4.2.3 Anti-bacterial activity assay

The Minimum Inhibitory Concentrations (MIC) method was used in this study to determine the antibacterial activity of crude extracts from the plant and bacterial endophytes. The following bacterial strains were used: *Bacillus cereus* (ATCC 10876), *Bacillus subtilis* (ATCC 19659), *Enterobacter aerogenes* (ATTC 13048), *Escherichia coli* (ATCC 10536), *Klebsiella pneumonia* (ATCC 10031), *Mycobacterium smegmatis* (ATCC 21293), *Mycobacterium marinum* (ATCC 927),

Proteus vulgaris (ATCC 33420), *Pseudomonas aeruginosa* (ATCC 10145), *Staphylococcus aureus* (ATCC 25923), *Streptococcus epidermidis* (ATCC 14990).

MICs were carried out according to the method outlined by Andrews, (2001) and Uche-Okereafor, (2016). The bacterial strains were inoculated into Mueller Hinton (MH) broth and allowed to grow overnight in an incubator at 37 ˚C for 24 to 36 hours depending on the growth rate of each bacteria and compared to a 0.5 McFarland's standard. The antibiotic, Streptomycin was used as the positive control and was prepared by weighing 0.032 mg in 1 mL of sterile distilled water while Dimethyl Sulfoxide (DMSO) was used as a negative control.

The crude secondary metabolite extracts from the identified endophytes were weighed (0.176 g) into empty autoclaved MacCourtney bottles to ensure sterility. The crude extracts were dissolved in DMSO (0.1 %) to make a stock solution of 32 mg/mL. Serial dilutions were carried out using the MH broth from 16 mg/mL down to 0.03125 mg/mL. The outer-wells of the plate were filled with sterile distilled water. Standardized overnight bacterial cultures (100 µL) were added into each well horizontally and vertically in 5 repeats for each bacterium. In vertical order, 100 µL of the diluted samples were added in the wells from 16 mg/mL down to 0.03125 mg/mL. The plates were covered and incubated overnight at 37 °C. Resazurin sodium salt solution (10 µL of 0.02 % (w/v)) was added to the wells and incubated for another two hours. Upon reduction, resazurin changes colour from blue to pink to clear, as oxygen becomes limiting within the medium, indicating metabolism. The well with a known concentration showing a slight color change, was used as MIC. The wells were visually inspected for color changes.

4.2.4 Anticancer activity assay

Crude extracts of *S. mauritianum* plant parts and secondary metabolites derived from bacterial endophytes were tested against two ATCC (Manassas, VA, USA) cancer cell lines: U87MG Glioblastoma and A549 Lung carcinoma cells for anticancer activity according to the methods of McCauley *et al*. (2013). The crude samples were weighed in Eppendorf tubes, 0.1 % DMSO was added and sonicated to aid dissolution, and a stock solution of 200 µg/mL was made. Serial dilutions were carried out using growth media from 100 µg/mL to 3.13 µg/mL. An MTS (3 (4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS)

(Promega, USA) *in-vitro* cytotoxicity assay was conducted to determine change in cell viability, through a colour change. An MTS compound (yellow) is metabolized by viable cells to form a dark purple-coloured compound, visible through UV Vis spectroscopy at 490 nm. The absorbance is directly proportional to the cell viability. The samples were analyzed in duplicates across three plates (n = 6) and the average value reported. The U87MG cells and A549 cells were grown using normal tissue culture techniques and 15% FBS addition. The cells (1×10^5 cells/mL) were incubated in 96 well plates at 37 ˚C overnight, with the subsequent addition of the supplied compounds, in concentrations of (100 µg/mL, 50.0 µg/mL, 25.0 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, and 0 µg/mL). The cells were left to incubate for 4 days, after which MTS (5 µL) was added to the cells. The absorbance values were measured at 490 nm after 1 h, 2 h, and 4 h incubation periods, averaged and the viability curves drawn up. Auranofin was used as a positive control based on its excellent activities against non-small cell lung cancer cells (Li *et al*., 2015; Roder and Thomson, 2015).

Cell viability was calculated using the following formulae:

$$\%\ \text{Cell Viability} = \frac{E_a - B_a}{C_a - B_a} \times 100$$

where E_a is absorbance of the extract, B_a is absorbance of the blank and C_a is the absorbance of the control (Handayani *et al*., 2018).

4.2.5 xCELLigence® real-time cell analyzer (RTCA) assay on U87MG cells

xCELLigence® RTCA assay was performed by initially seeding 1 x 10^5 cells/mL of U87MG cells on gold microelectrode precoated 96 well electronic plates (E-Plate® 96; ACEA Biosciences Inc., San Diego, CA, USA) and incubating at 37 °C in 5 % CO_2 atmosphere as previously described by Sun *et al*., (2016). Crude extracts of *S. mauritianum* ripe fruit cover was dissolved in DMSO were then added at concentrations of (100 µg/mL, 50.0 µg/mL, 25.0 µg/mL, 12.5 µg/mL, 6.25 µg/mL and 3.125 µg/mL) in triplicates. Cells were then left to incubate for a further 96 hours, with impedance measurements taken every 15 minutes during the incubation period. The data was retrieved, and a graphic representation of the toxicity was constructed.

4.2.6 Statistical analysis

The effects of the samples on cell viability based on the different concentrations (100 – 3.13 µg/mL) were analysed. The quantitative variables were analyzed in the software package IBM SPSS Statistics 26. Analysis of Variance (ANOVA) and post hoc were used to analyze the means ± standard deviation (SD) of different extracts at various concentrations. The difference in cytotoxicity estimate was statistically significant at $P \leq 0.05$.

4.3 RESULTS AND DISCUSSIONS

Antibacterial assay

Minimum inhibitory concentration of crude extracts from both *S. mauritianum* plant parts and endophytes ranged from 16.00 mg/mL to 0.031 mg/mL. Several samples showed inhibition at a concentration of 0.125 mg/mL showing potential for development into compounds with promising bioactivities against pathogenic microorganisms. The results of the antibacterial study done using *S. mauritianum* endophytes and plant crude extracts are summarized in Tables 4.1 and 4.2 respectively where it is seen that *Arthrobacter* sp. had the most notable activity against the test bacterial pathogens with MIC ranging from 0.063 to 1.000 mg/mL while for plant parts the green fruits had the most significant activity with MIC range of 0.031 – 1.000 mg/mL against test bacterial pathogens.

Table 4.1. Results of the antibacterial activity test carried out on the crude secondary metabolites extracted from the identified endophytes from *Solanum mauritianum*

Test organism	Gram reaction	NU 01 (Pa)	NU 02 (Xs)	NU 03 (Pe)	NU 04 (Bs)	NU 05 (Pv)	NU 06 (Bl)	NU 07 (As)	Positive control
					MIC (mg/mL)				(µg/mL)
B. cereus	Positive	**0.125**	**0.500**	**0.500**	**0.250**	**0.500**	**0.250**	**0.063**	0.031
B. subtilis	Positive	**0.250**	**0.500**	**0.500**	**0.500**	**0.125**	**0.250**	**0.125**	0.031
E. aerogenes	Negative	4.000	4.000	4.000	8.000	8.000	1.000	**0.500**	0.125
E. coli	Negative	**0.500**	16.00	**0.500**	8.000	1.000	1.000	**0.250**	0.125
K. pneumoniae	Negative	2.000	4.000	4.000	**0.500**	8.000	2.000	**0.500**	0.125
M. marinum	Positive	**0.125**	8.000	**0.500**	**0.125**	**0.500**	**0.500**	**0.250**	0.063
M. smegmatis	Positive	**0.125**	16.00	**0.125**	**0.125**	**0.125**	**0.500**	1.000	0.063
P. vulgaris	Negative	**0.500**	4.000	4.000	1.000	4.000	1.000	**0.500**	0.125
P. aeruginosa	Negative	1.000	2.000	2.000	**0.500**	4.000	1.000	**0.250**	0.125
S. aureus	Positive	**0.063**	8.000	**0.125**	**0.125**	**0.125**	**0.063**	**0.063**	0.031
S. epidermidis	Positive	**0.125**	4.000	**0.125**	**0.500**	**0.500**	**0.500**	**0.125**	0.063

Note: NU 01 Pa = *P. ananatis*, NU 02 Xs = *Xanthomonas* sp., NU 03 Pe = *P. eucalypti*, NU 04 Bs = *B. safensis*, NU 05 Pv = *P. vagans*, NU 06 Bl = *B. licheniformis*, NU 07As = *Arthrobacter* sp., Positive control = Streptomycin

Table 4.2. Results of the antibacterial activity test carried out on the crude secondary metabolites extracted from *Solanum mauritianum* plant part.

Test organism	Gram reaction	Ripe fruit (coat)	Stem	Ripe fruit (seeds)	Unripe/Green fruit	Leaves	Positive control
				MIC (mg/mL)			(µg/mL)
B. cereus	Positive	**0.063**	**0.125**	**0.250**	**0.063**	**0.125**	0.031
B. subtilis	Positive	**0.125**	**0.125**	**0.500**	**0.031**	**0.063**	0.031
E. aerogenes	Negative	**0.500**	2.000	2.000	**0.500**	2.000	0.125
E. coli	Negative	**0.500**	**0.500**	4.000	**0.500**	4.000	0.125
K. pneumoniae	Negative	1.000	**0.500**	1.000	**0.500**	**0.500**	0.125
M. marinum	Positive	**0.500**	**0.500**	**0.250**	**0.125**	**0.125**	0.063
M. smegmatis	Positive	**0.500**	**0.125**	**0.500**	**0.125**	**0.500**	0.063
P. vulgaris	Negative	1.000	**0.500**	1.000	1.000	4.000	0.125
P. aeruginosa	Negative	**0.500**	**0.125**	**0.500**	**0.031**	4.000	0.125
S. aureus	Positive	**0.125**	**0.063**	**0.125**	**0.125**	1.000	0.031
S. epidermidis	Positive	**0.500**	**0.125**	1.000	**0.125**	**0.125**	0.063

Though there are many studies on endophytes and their importance in plants, most especially fungal endophytes, endophytes (mostly bacterial endophytes) are still underexplored as potential sources of novel biologically active compounds that can be used for medical, industrial and commercial purposes. With many of these endophytes being found in virtually all higher plants, growing in different areas under diverse environmental factors, the wealth of possibilities that exist in the exploitation of these endophytes for their potential usefulness is quite exciting (Mehanni and Safwat, 2010). It has been reported by van Vuuren, (2008) that with regards to minimum inhibitory concentration experiments using crude extracts, MIC values of/below 1 mg/mL are considered noteworthy.

P. ananatis – NU 01 extracts had significant MIC values of between 0.063 – 4.000 mg/mL. The most significant MIC value of 0.063 mg/mL was seen on pathogenic *Staphylococcus aureus*. Other notable inhibitory activities were seen on *B. cereus, M. marinum, M. smegmatis, S. epidermidis* with an MIC value of 0.125 mg/mL and *B. subtilis* with a MIC of 0.250 mg/mL. Antimicrobial activity of a bioactive compound from *P. ananatis* have been reported in a previous study by Pomini *et al*. (2006) which could support the notable inhibitory activity of its crude secondary metabolites in this study.

P. vagans – NU 05 extracts had MIC values ranging from 0.125 - 8.000 mg/mL. The most susceptible microbes were *B. subtilis, M. smegmatis* and *S. aureus* with a MIC value of 0.125 mg/mL. A notable MIC value of 0.500 mg/mL was also observed for *B. cereus* and *S. epidermidis*. A peptide present in *P. vagans* has been reported to possess antibacterial activity (Kamber *et al*., 2012), this could serve as a justification for the antibacterial activity seen from the crude secondary metabolites extracts of *P. vagans*.

P. eucalypti – NU 03 extracts showed MIC values between 0.125 – 4.000 mg/mL. Noteworthy inhibition was observed for *M. smegmatis, S. aureus* and *S. epidermidis* with a MIC value of 0.125 mg/mL. Other significant inhibitions were seen on *B. cereus, B. subtilis, E. coli* and *M. marinum* with a MIC value of 0.500 mg/mL. This, to the best of our knowledge is an initial report on the antibacterial activity of secondary metabolites from *P. eucalypti*.

B. safensis – NU 04 had MIC values between 0.125 - 8.000 mg/mL and the most susceptible microbes were *B. cereus, B. subtilis, K. pneumonia, M. marinum, M. smegmatis, P. vulgaris, P. aeruginosa. S. aureus* and *S. epidermidis* all with MICs below 1 mg/mL. *Bacillus licheniformis* – NU 06 had MIC values between 0.063 – 2.000 mg/mL. It was active against all tested microbes with MIC of/below 1 mg/mL except for *K. pneumonia* with a 2 mg/mL MIC. Many *Bacillus* species, for examples: *B. subtilis, B. polymyxa, B. brevis, B. licheniformis, B. circulans, B. safensis, B. cereus* are known to produce antimicrobial substances (Yilmaz *et al*., 2006), (Mayer and Kronstad, 2017) this validates the antibacterial activity observed for *B. safensis* and *B. licheniformis* crude extracts seen in this study.

Xanthomonas sp. – NU 02 extracts when compared to other endophytes in this study had the least significant MIC values of between 0.500 – 16.00 mg/mL. Noteworthy MIC value of 0.500 mg/mL was observed for pathogenic *B. cereus B. subtilis and S. aureus*. As far as we know, this is an initial report on the antibacterial activity of *Xanthomonas* sp. *Xanthomonas* sp. and *Luteibacter* sp. both from the order: Xanthomonadales and family: Rhodanobacteraceae are closely related. Although, as far as we know, there is no record of antibacterial activity of *Xanthomonas* sp., *Luteibacter* sp. has been reported to be produce fatty acids that are active against a range of pathogenic microbes (Barthélemy *et al*., 2019).

Arthrobacter sp. – NU 07 had the most significant activity among all endophytes. Its extracts had MIC values ranging from 0.063 - 1.000 mg/mL and was active against all test organisms. Arthrobacter sp. have previously been reported to possess antibacterial activity against *Dietzia* sp., *Micrococcus flavus, B. subtilis, S. aureus, B. cereus, M. luteus, E. coli, P. aeroginosa,* and *S. typhi* (Pathak *et al*., 2011). Various organisms of Phylum: Actinobacteria which includes *Arthrobacter* spp. have previously been reported to possess antibacterial activity against a wide range of pathogenic microbes (Villarreal-Gómez *et al*., 2010; Leiva *et al*., 2015) which supports our findings.

The stem of *S. mauritianum* which was the host of all the isolated *Pantoea* spp. endophytes was analyzed for antibacterial activity. The MIC values seen for the stem crude extracts ranged from 0.063 – 2.000 mg/mL. The crude extract showed very significant activity against *S. aureus* with

MIC value of 0.063 mg/mL which is also the same MIC seen on *S. aureus* when *P. ananatis* secondary metabolites were tested against it. There was activity seen for *B. cereus, B. subtilis, M. smegmatis, P. aeruginosa* and *S. epidermidis* with MICs of 0.125 mg/mL. MIC of 0.500 mg/mL was seen for *E. coli, K. pneumonia, M. marinum* and *P. vulgaris*.

The ripe fruits, green fruits and leaves of *S. mauritianum* were also analyzed for antibacterial activity and the green fruits were the most active against all test organisms. The MIC values seen for the ripe fruit seeds crude extracts ranged from 0.125 – 4.000 mg/mL, ripe fruit cover crude extracts from 0.063 – 1.000 mg/mL, leaves from 0.063 – 4. 000 mg/mL and the green fruits from 0.031 – 1.000 mg/mL. Several *Solanum* species have been reported to have antibacterial activities against *S. aureus, E. coli, P. aeruginosa, B. subtilis* and other common bacterial pathogens (Chah *et al*., 2000; Aliero and Afolayan, 2006; Koduru *et al*., 2006; Niño *et al*., 2006; Rana *et al*., 2016) these reports validate the results on the antibacterial activity of the *S. mauritianum* plant parts reported in this study.

Antibiotics or hydrolytic enzymes can be released by endophytes to prevent colonization of microbial plant pathogens (Strobel, 2003; Berg and Hallmann, 2006), or prevent insects (Azevedo *et al*., 2000) and nematodes (Hallmann *et al*., 1998) from infecting plants. In other cases, endophytes release metabolites which activate host defense mechanism against other pathogenic organisms, in a process known as induced systemic resistance (Kloepper and Ryu, 2006). The most abundant metabolite producing Gram-positive bacterial endophytes found in various environments are *Bacillus* and *Streptomyces* species (Reinhold-Hurek and Hurek, 2011; Frank *et al*., 2017; Ek-Ramos *et al*., 2019).

In this study, crude secondary metabolites from both plant part and isolated endophytes showed notable antibacterial activities against pathogenic microbes. Antimicrobial activity of compounds produced by plant endophytes have previously been reported (Huang *et al*., 2001; Li and Strobel, 2001). It has been reported that endophytes are the chemical synthesizers within plants. Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against pathogens and some of these compounds have been reported to be useful for drug discovery (Guo *et al*., 2008; Sadrati *et al*., 2013). This supports the inhibitory activities seen in both the plant part

analyzed and the isolated endophytes. It can also be seen from the results that the Gram-positive bacteria were more susceptible to both plant and endophytes crude extracts than the Gram-negative bacteria. This could be due to the presence of the outer membrane in the Gram-negative organisms which excludes certain drugs and antibiotics from penetrating the cell; this in part accounts for why Gram-negative bacteria are generally more resistant to antibiotics than the Gram-positive bacteria (Salton and Kim, 1996). The results from this study suggests that the secondary metabolites from *S. mauritianum* plant and its bacterial endophytes could be explored further for development of new pharmaceutical products against pathogens.

Anticancer assay

The extracts showed no notable anticancer activity against the glioblastoma cells as seen in Figure 3. While there was a reduction in cell viability of the lung carcinoma cells (as shown in figures 4.1 – 4.4) when treated with some of the samples, they could not be considered notable as they were less than 50 % reduction. Statistically significant differences of the effects the various samples (plant part and bacterial endophytes) had on UMG87 glioblastoma cells and A549 lung carcinoma cells was observed at $P \leq 0.05$ level. Cell viability under concentrations 100 – 3.13 µg/mL ranged from 62.0582 – 308.9493 % across all samples of plants and endophytes for the glioblastoma cells; and from 67.1244 - 135.4234 % across all samples of the lung carcinoma cells.

The most significant activity of 37.9417 % at 100 µg/mL was observed for the glioblastoma cells treated with *S. mauritianum* ripe fruit coat (figure 4.1) and the xCelligence analysis of *S. mauritianum* ripe fruit coat revealed that the sample had a concentration specific toxicity (figure 4.5), with possible metabolism of the toxin. The sample seemed to have toxicity centered around the cell membrane.

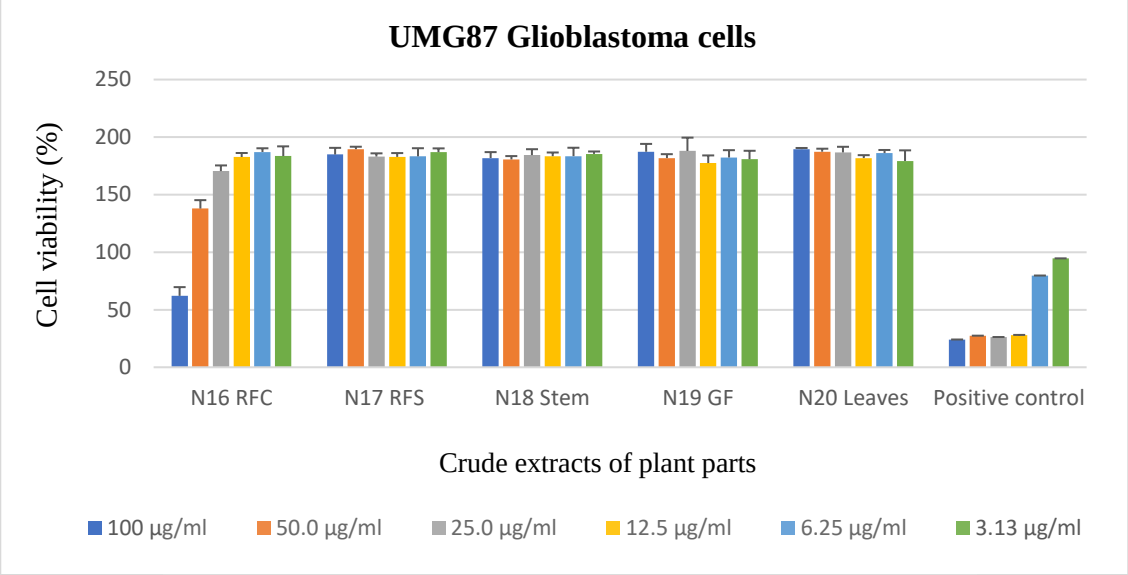

Figure 4.1: Cytotoxic effects of secondary metabolites from Solanum mauritianum and the plant on UMG87 glioblastoma cells tested at different concentrations ranging from 100 – 3.13 µg/mL. Auranofin was used as positive control. N16 = Ripe fruit coat, N17 = Ripe fruit seed, N18 = Stem N19 = Green fruit N20 = Leaves.

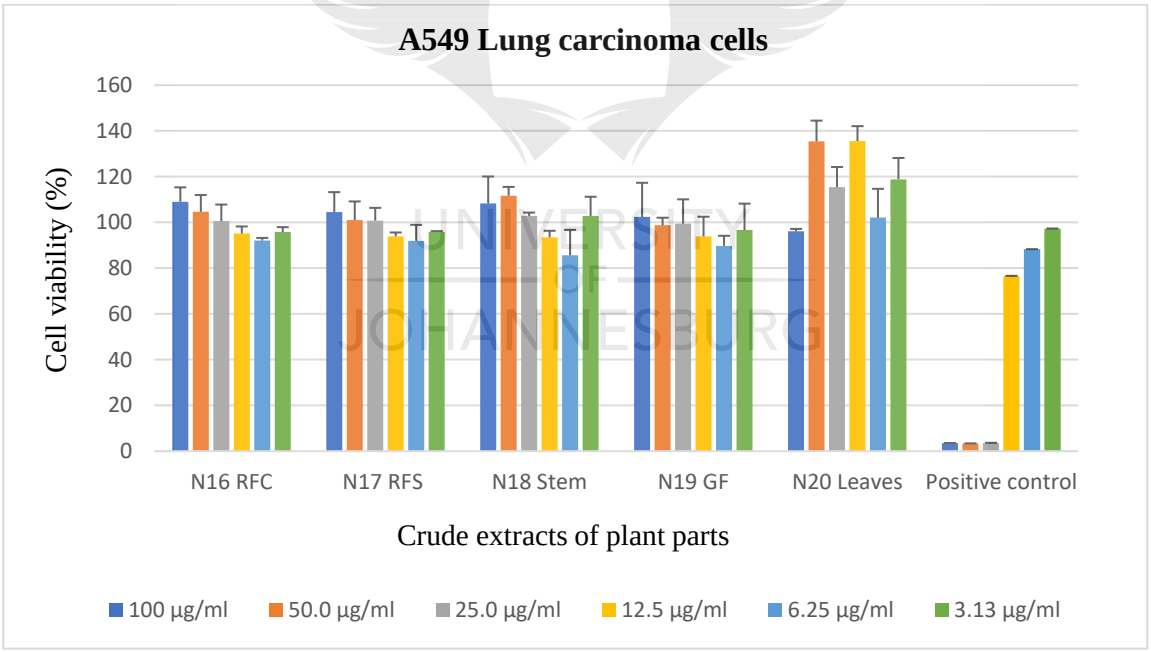

Figure 4.2: Cytotoxic effects of secondary metabolites from Solanum mauritianum and the plant on A549 Lung carcinoma cells tested at different concentrations ranging from 100 – 3.13 µg/mL. Auranofin was used as positive control. N16 = Ripe fruit coat, N17 = Ripe fruit seed, N18 = Stem N19 = Green fruit N20 = Leaves.

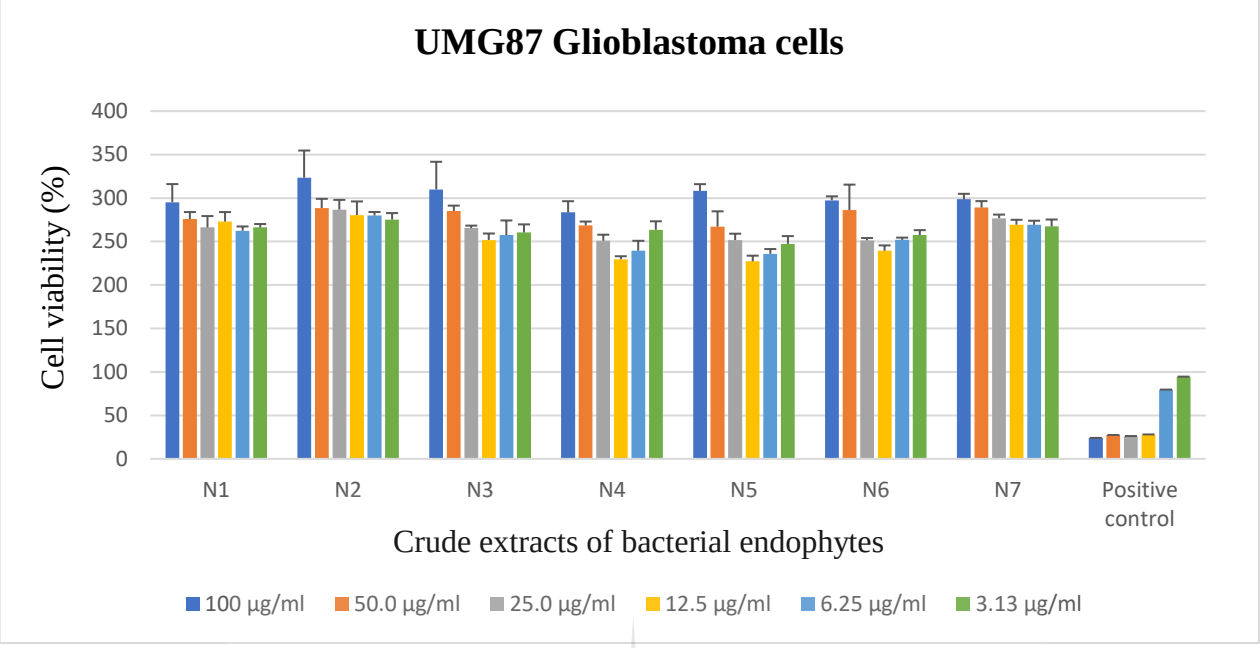

Figure 4.3: Cytotoxic effects of secondary metabolites of bacterial endophytes from Solanum mauritianum on UMG87 glioblastoma cells tested at different concentrations ranging from 100 – 3.13 µg/mL. Auranofin was used as positive control. N1 = P. ananatis, N2 = Xanthomonas sp., N3 = P. eucalypti, N4 = B. safensis, N5 = P. vagans, N6 = B. licheniformis, N7 = Arthrobacter sp.

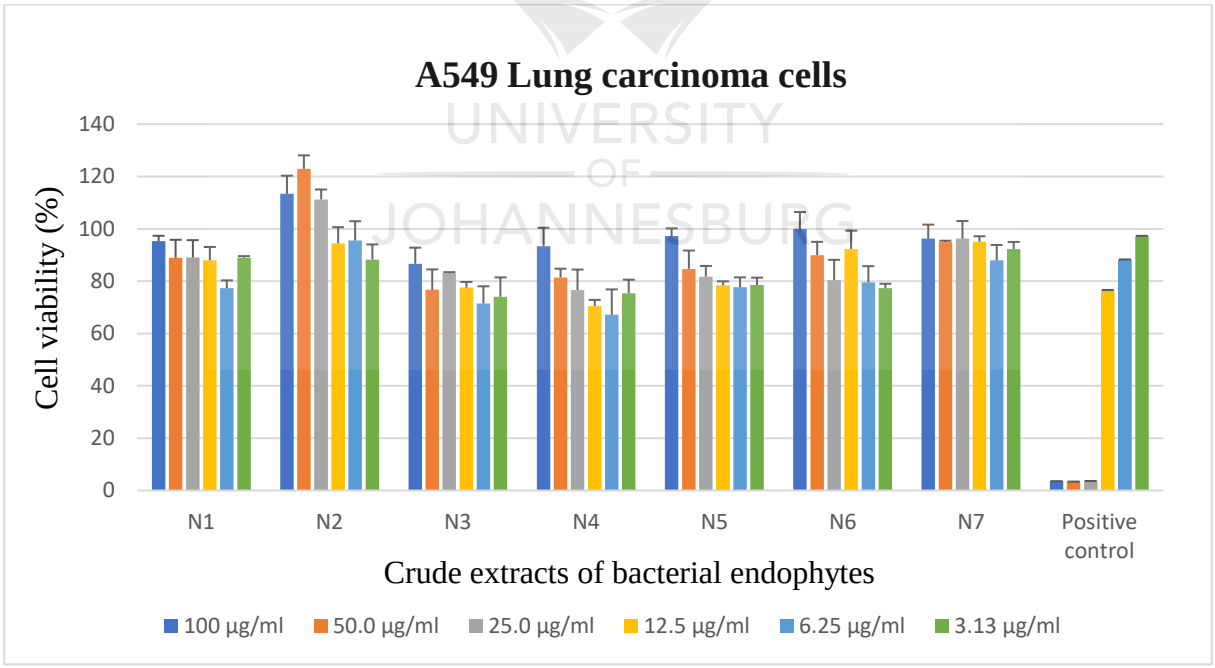

Figure 4.4: Cytotoxic effects of secondary metabolites of bacterial endophytes from Solanum mauritianum on A549 Lung carcinoma cells tested at different concentrations ranging from 100 – 3.13 µg/mL. Auranofin was used as positive control. N1 = P. ananatis, N2 = Xanthomonas sp., N3 = P. eucalypti, N4 = B. safensis, N5 = P. vagans, N6 = B. licheniformis, N7 = Arthrobacter sp.

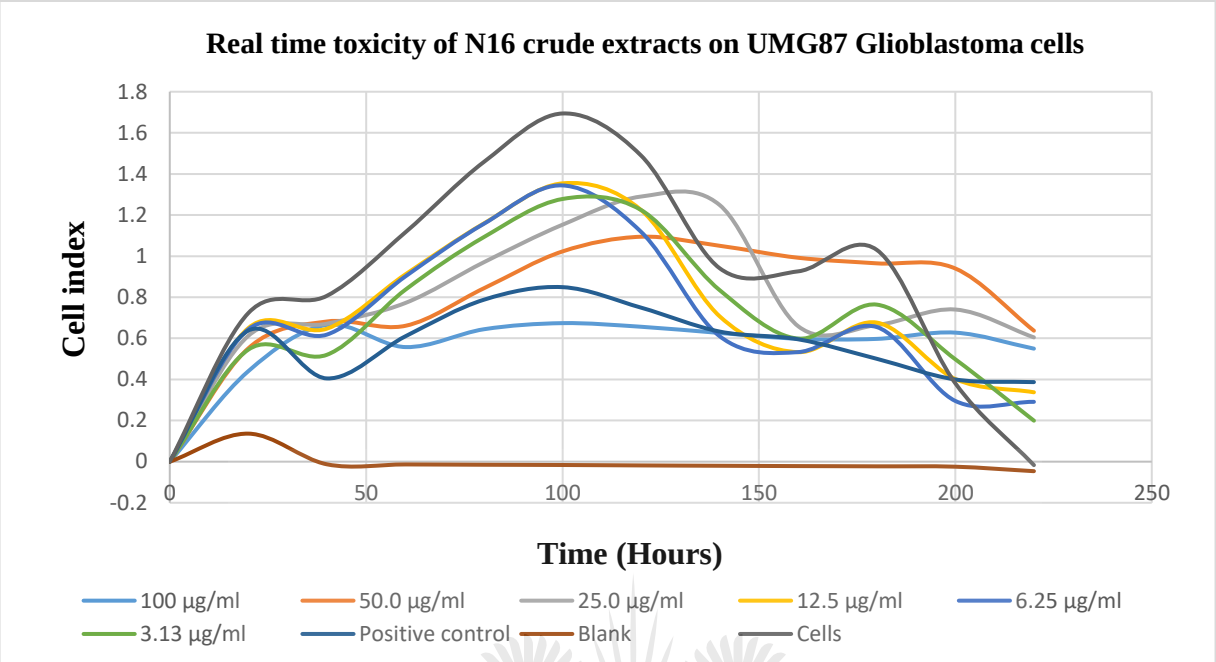

Figure 4.5: RCTA assay of N16 - Ripe fruit coat extract of S. mauritianum on UMG87 glioblastoma cells. Six concentrations ranging from 100 µg/mL – 3.13 µg/mL were administered on the cells. Cells were monitored up until the 220th hour on the timeline. Cell viability was recorded as cell index, which is a relative change in measured impedance.

Crude extracts of plant and endophytes in this study were tested against two known resistant human cancer cells – glioblastoma and lung carcinoma cells. Based on the US National Cancer Institute (NCI) guidelines, a crude extract is generally considered to have cytotoxic activity if the IC_{50} value is ≤20 µg/mL, following incubation between 48 and 72 hours (Ghareeb *et al*., 2014; Alabsi *et al*., 2016).

The most promising activity was observed for sample N16 (*S. mauritianum* ripe fruit cover) against glioblastoma cell. While cell viability was not reduced to the recommended ≥50 %, the extract reduced cell viability by 37.94 % which is the highest across all samples (both plant and endophyte extracts). Although there were no noteworthy anticancer activities of the analyzed crude extracts against the cancer cells, some samples showed promising inhibitory effects on cell viability. At 100 µg/mL, a reduction in cell viability for samples N1, N3, N4, N7 and N20 was observed. N1 reduced cell viability by 4.69 %, N3 by 13.34 %, N4 by 6.61 %, N7 by 3.77 % and N20 by 3.92 %. At concentration 50 µg/mL, there was decrease in cell viability across all samples except for N2, N16

- 18 and N20. N1 reduced viability by 11.05 %, N3 by 23.33 %, N4 by 18.59 %, N5 by 18.74 % N6 by 10.06 %, N7 by 5.03 % and N19 by 1.28 %. Concentration 25 µg/mL showed inhibitory activity on cell viability for samples N1, N3 – N7, reducing cell viability by 10.98 %, 16.97 %, 23.36 %, 18.25 %, 19. 64 % and 3.74 % respectively but samples N2, N16 – N20 showed no reduction in cell viability. Concentration 12.5 µg/mL reduced cell viability across all samples, except for sample N20. N1 reduced cell viability by 12.30 %, N2 by 5.53 %, N3 by 22.47 %, N4 by 29.36 %, N5 by 21.61 %, N6 by 7.70 %, N7 by 4.95 %, N16 by 4.86 %, N17 by 6.10 %, N18 by 6.51 % and N19 by 6.12 %. At concentration 6.25 µg/mL, samples N1 – N19 showed a decrease in cell viability, the cells were reduced by 22.67 % for N1, 4.50 % for N2, 28.51 % for N3, 32.87 % for N4, 22.3167 % for N5, 20.41 % for N6, 10.07 % for N7, 7.91 % for N16, 8.09 % for N17, 14.39 % for N18 and 10.25 % for N19. Reduction in cell viability was not observed for sample N20. At concentration 3.13 µg/mL, samples N18 and N20 showed no reduction in cell viability but samples N1 – N7 reduced cell viability by 10.99 %, 11.75 %, 25.95 %, 24.58 %, 21.47 %, 22.71 %, 7.73 % respectively and samples N16, N17 and N19 reduced viability by 4.21 %, 4.16 % and 3.37 % respectively.

Although it has been previously indicated that different plant parts in some other species of *Solanum* possess anticancer properties: *S. nigrum* leaves possess anticancer properties against Ehrlich ascites carcinoma cell (EACC) line and Hepatoma cell (HepG2) line (Aboul-Enein *et al*., 2014), tomatidine and solasodine from *S. aculeastrum* were shown to have inhibitory effect on HT-29 (colonic adenocarcinoma), HeLa (cervical carcinoma), and MCF-7 (breast adenocarcinoma) cells (Koduru *et al*., 2007), as far as we know, this is a first report on anticancer study involving *S. mauritianum*. To the best of our knowledge there is no previous report on the anticancer properties of any of the *Pantoea* endophytes reported in this study, but exopolysaccharides isolated from an endophyte: *Bacillus amyloliquefaciens* showed anticancer activity against gastric carcinoma cell lines (Chen *et al*., 2013). Other studies on anticancer properties of fungal endophytes have also been previously reported elsewhere (Cui *et al*., 2011; Lakshmi and Selvi, 2013; Wu *et al*., 2015). It is reported that Glioblastoma (GB) and lung cancer are among the most lethal human cancers. Glioblastoma (GB) tumor cells as well as lung cancer cells have been shown to exhibit drug resistance and are highly infiltrative (Shanker *et al*., 2010; Noch *et al*., 2018; Atkins *et al*., 2019) and this resistance could account for the results observed in this study. Resistance to treatment and poor survival have been

attributed to the presence of cancer stem cells (CSCs) in GB (Bradshaw *et al*., 2016; van Schaijik *et al*., 2018) and in lung carcinoma cells (Tsvetkova and Goss, 2012).

Statistical analysis of the samples used against both glioblastoma and lung carcinoma cells based on the concentrations (100 – 3.13 µg/mL) was found to be non-significant as *P* was greater than 0.05.

In the Glioblastoma cells and the Lung carcinoma cells, there was no significant difference in the 12 samples (plants and endophytes) based on their effect on cell viability. The statistical analysis on GB cells according to ANOVA data $F(5, 72) = 0.097$, showed that *P* is 0.992 which is higher than 0.05. Post hoc testing revealed no significant differences in the samples concentration dependent cell viability with 100 µg/mL (M = 226.6247,SD = 98.3059), 50 µg/mL (M = 220.3589, SD = 78.8807), 25 µg/mL (M = 214.4514, SD = 70.2561), 12.5 µg/mL (M = 208.2261, SD = 66.3912) 6.25 µg/mL (M = 215.2182, SD = 55.2228733) and 3.13 µg/mL (M = 219.0663, SD = 54.5030). In the Lung carcinoma cells, ANOVA data showed $F(5, 72) = 0.216$, and $P = 0.955$. The samples had no significant difference in their effects on cell at varying concentrations 100 µg/mL (M = 92.7544, SD = 27.7697699), 50 µg/mL (M = 91.8759,SD = 31.3419), 25 µg/mL (M = 87.751713, SD = 28.0179607), 12.5 µg/mL (M = 91.128037, SD = 15.9153) 6.25 µg/mL (M = 85.1010, SD = 9.9409) and 3.13 µg/mL (M = 90.9153, SD = 12.6310).

xCELLigence® real-time cell analyzer (RTCA) assay on U87MG cells

UMG87 glioblastoma cells were exposed to N16 (RFC) extracts for 216 hours and the response was monitored using RTCA. The toxicity of N16 extract was not concentration dependent and appeared to reduce the cell viability initially. Although the cells did not recover fully, it followed the same growth pattern as the untreated cells, illustrating that the toxin had possibly been metabolized and was causing no further toxicity to the cells.

4.4 CONCLUSION

This study investigated the capability of the *S. mauritianum* extracts and that of its endophytes for antibacterial and anticancer activity. The results obtained from this study clearly showed that *S. mauritianum* and its bacterial endophytes had noteworthy antibacterial activity against a range of

pathogenic bacteria. The ripe fruits coat of *S. mauritianum* showed also showed a promising dose dependent cytotoxic activity against UMG87 glioblastoma cells. The activities could further be studied by identifying the compounds responsible for this antibacterial activity and further exploring them for the treatment of infections caused by common resistant bacterial pathogens like *E. coli* and *S. aureus* and also for the treatment of cancers.

It is important to note that while the endophytes and their host plant (*S. mauritianum*) had noteworthy antibacterial activities against a range of pathogenic bacteria, they also failed to reduce cell viability of both glioblastoma and lung carcinoma cells significantly. This supports the third hypothesis of this study that endophytes and their host plants tend to have similar biological activities.

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CHAPTER FIVE

UNTARGETED SECONDARY METABOLITE PROFILING OF CRUDE EXTRACTS FROM ISOLATED BACTERIAL ENDOPHYTES AND *Solanum mauritianum* Scop. PLANT PARTS USING LIQUID CHROMATOGRAPHY COUPLED TO QUADRUPOLE TIME-OF-FLIGHT WITH TANDEM MASS SPECTROMETRY (LC-QTOF-MS/MS).

5.0 ABSTRACT

Microorganisms are known to produce a plethora of secondary metabolites. Endophytes tend to produce the same compounds or groups of compounds as their host plant, and other compounds which are different from what is produced by the host plant. The aim of this study was to screen isolated bacterial endophytes from *S. mauritianum* for the presence of bioactive secondary metabolites. The crude endophytic and plant extracts were analyzed using liquid chromatography coupled to quadrupole time-of-flight with tandem mass spectrometry (LC-QTOF-MS/MS) and the observed *m/z* values and fragments identified on MetFrag. A total of 106 bioactive compounds belonging to diverse phytochemical groups were identified in both crude extracts of plant and bacterial endophytes. 46 of the compounds seen in the plants alone of which 43.8 % were alkaloids, 38 compounds in the endophytes alone with 36.7 % of those compounds being alkaloids. The following 11 compounds: anatabine, swainsonine, neoquassin, genipin, droserone, loganin, hetisine, columbin, tuliposide A, montanol and cannabielsoin were seen in both plants and endophytes. Both the plants and endophytes were rich in alkaloids based on the high number of alkaloidal compounds identified in them. Other groups of secondary metabolites identified include flavonoids, terpenoids, coumarins, lignans, glycosides and chromones. While most of the identified compounds identified in this study have been reported in other plants and microbes, this is the first study reporting the presence of these compounds in bacterial endophytes isolated from *S. mauritianum*. This study opens up the opportunity for further isolation and purification of the compounds identified in this study for pharmacological uses.

Keywords: Endophytes, LC-QTOF-MS/MS, Secondary metabolites, *Solanum mauritianum*.

Results from this chapter have been submitted to in the *Transactions of the Royal Society of South Africa* and *Journal of Tropical Medicine* for publication.

5.1 INTRODUCTION

The requirement for new and valuable chemical compounds to help and offer alleviation in all parts of the human condition is ever-developing. Most plants produce hundreds if not a huge number of novel compounds as an adjustment to their condition with the end goal of self-protection and cooperation with different living beings in the earth; these are aggregately alluded to as plant secondary metabolites, or natural products (Salam and Quave, 2018). The resistance of microorganisms to drugs, the appearance of dangerous infections caused by viruses, the steady issues of sicknesses in people with organ transplants, and the huge increment in the rate of contaminations in the total populace all feature our inadequacy to adapt to these clinical issues. Notwithstanding these troubles confronting humanity are loss of biodiversity, environmental degradation and waste of land and water (Strobel *et al*., 2004). This makes the need to safeguard the earth and biodiversity apparent notwithstanding finding new wellsprings of natural products or secondary metabolites to address the ever-developing health care needs.

Among every single known maker of small molecule natural products, microorganisms speak to a rich wellspring of bioactive metabolites that find wide-extending applications as agrochemicals, antimicrobials, antiparasitics, anticancer agents, etc. (Gunatilaka, 2006). Microorganisms can use different strong substrates as an outcome of their decent variety in organic and biochemical advancement; the strong substrates used by microorganisms incorporate, among others, live plants. Fungi and bacteria are known to team up with numerous plants to shape commonly useful (mutualistic) affiliations; these organisms are classified "endophytes". The term endophyte alludes to fungi or bacterial species that colonizes inside organs of plants however does not affect its host(s) in a pathogenic way (Nisa *et al*., 2015). In their symbiotic affiliation, the host plant (macrophyte) ensures and takes care of the endophyte, which consequently produces bioactive metabolites to upgrade the development and competitiveness of the host and to shield it from herbivores and plant pathogenic microorganisms (Dreyfuss and Chapela, 1994; Gunatilaka, 2006).

Plants produce an assorted exhibit of in excess of 100,000 secondary metabolites, and can be ordered based on composition, their synthesis pathway, or chemical structure (Qin *et al*., 2011). There are reports about plants and their colonizing endophytes delivering same or comparative secondary metabolites or classes of secondary metabolites (Tan and Zou, 2001; Zhang *et al*., 2006;

Martinez-Klimova *et al*., 2017). A basic grouping of these secondary metabolites incorporates three principle classes: i. phenolic mixes, which are produced using basic sugars, containing benzene rings, oxygen and hydrogen; ii. terpenoids, which are produced using mevalonic acid and made primarily out of hydrogen and carbon; and iii. alkaloids, which are nitrogen-containing compounds (Nazir *et al*., 2011; Wang *et al*., 2012). Be that as it may, extracting, isolating and purifying of secondary metabolites from natural sources is a difficult methodology inferable from complex science and isolation strategies to get bioactive compounds from natural products. Extraction of metabolites from endophytes is influenced by different elements, for example, the season when the sample was collected, climatic condition and topographical area (Shukla *et al*., 2014). Nevertheless, with a progressive engineered process that has been created during the previous years, extraction from plants and other characteristic sources has now gotten increasingly possible, effective and expedient (Hussain *et al*., 2012).

In this study, we had hypothesized that plants and their endophytes produce similar compounds/ group of compounds and as such the latter can be exploited in place of plants in search of these beneficial compounds, to reduce the threat to biodiversity and ensure conservation. The untargeted secondary metabolite screening of *S. mauritianum* and its bacterial endophytes was done to validate our hypothesis.

5.2 MATERIALS AND METHODS

5.2.1 Untargeted secondary metabolite profiling of active crude extract by LC-QTOF-MS/MS.

Secondary metabolite profiling of the endophytic bacteria and plant crude extracts were investigated using Liquid chromatography coupled to quadrupole time-of-flight with tandem mass spectrometry (LC-QTOF-MS/MS), according to the exact method described by Tapfuma *et al*., (2019a, 2019b). All instrumental conditions and data analysis were done using the same methods reported by Tapfuma *et al*., (2019a, 2019b).

5.2.1.1 Extraction of Crude metabolites from plant and bacterial endophytes

The crude secondary metabolite extracts from aerial parts (ripe and unripe fruits, stem, leaves) of *Solanum mauritianum* and their bacterial endophytes (*Pantoea ananatis*, *Xanthomonas* sp.,

Pantoea eucalypti, Bacillus safensis, Pantoea vagans, Bacillus licheniformis, Arthrobacter sp.) were analyzed to identify the compounds present in the extracts.

The endophytic bacteria were cultured by inoculating them into 3 Litres nutrient broth (contained in 7 different 5 Liter Schott bottles) and incubated at 27 °C for 7 days while constantly being shaken at 200 rcf. After the 7 days incubation period, 60 g/L of sterile mesh resin Amberlite® XAD7HP 20-60 (Sigma-Aldrich, SA) was added and the resin and culture mixture was incubated and shaken for 2 hours at 200 rcf.

The resin from each sample was filtered using sterile cheesecloth and organic compounds adsorbed by the resin were washed off the resin using acetone. The acetone fraction was concentrated using a rotary evaporator. Liquid-liquid extraction was done using ethyl acetate 1:1 (v/v) to extract the crude secondary metabolites from the acetone concentrate. The solvents were removed using a rotary evaporator. The extracts were stored in clean sterile amber bottles before analysis.

The aerial parts of *Solanum mauritianum* were harvested, washed, dried and blended to fine powder. The blended plant samples were then weighed (200 g) into 2 Litres (contained in 5 Litre Schott bottles) of chloroform/methanol (50:50 v/v) mixture. The mixtures were allowed to stand on a platform shaker for 24 hours, after which they were filtered through a Whatman's No.1 filter paper and the solvents were evaporated using a rotary evaporator at 40 ˚C. The process repeated twice to guarantee the efficient extraction of all necessary compounds. The crude extracts were collected in amber bottles, placed in a desiccator for complete dying stored till analysis was done.

5.2.1.2 Sample preparation

The crude endophytic bacteria and plant extracts were prepared for LC-QTOF-MS/MS analysis by adding 1 mg/mL (w/v) of the sample in HPLC grade methanol (Merck, Johannesburg South Africa). The mixture was sonicated for 8-10 mins until the crude extracts were fully dissolved. The dissolved samples were afterward filtered via a 0.22 µm polyvinylidene fluoride (PVDF) syringe filter into 1 mL LC-MS auto sampler vials. Same extraction process was repeated for nutrient agar used to grow the bacterial endophyte, representing the blank..

5.2.2 Instrument conditions

The LC-QTOF system used in this study was a Dionex UltiMate 3000 Ultra-high-performance liquid chromatography (UHPLC;Thermo Scientific, Darmstadt, Germany) that was attached to a Compact™ QTOF (Bruker Daltonics, Bremen, Germany) which uses an electrospray ionization (ESI) interface. In the system, 5 µL injection volume was used for chromatographic separation of analytes in reverse phase ultra-high-performance liquid chromatography (RP-UHPLC) through a Raptor ARC-18 column (Restek, Bellefonte, PA, USA) with the following dimensions: particle size as 2.7 µm, length as 100 mm, internal diameter as 2.1 mm and pore size as 90 Å. HyStar software version 2.10 (Thermo Scientific, Darmstadt, Germany) was utilized for instrument operation, control and data acquisition.

The mobile phases were; solvent A: 0.1 % formic acid in water (H_2O;v/v) and solvent B: 0.1 % formic acid in acetonitrile (v/v) were used as the mobile phase. The initial gradient flow of the mobile phase was 2.0 min isocratic step at 5 % which was followed by an increase to 95 % in 28 min, isocratic step at 95 % for 5 min then followed by a decrease to 5 % B in 1 min upon re-equilibration to the initial conditions at flow rate of 300 µL/min. The total run time for the analysis was set at 40 mins. The following ESI(+) parameters were set: dry heater temperature at 220 °C; set capillary voltage at 4.5 kV; end plate offset at − 500 V; nebulizer gas pressure at 1.8 Bar and dry gas flow rate at 2.5 L/min. The mass spectra were attained in centroid mode range of 50 – 1300 *m/z*.

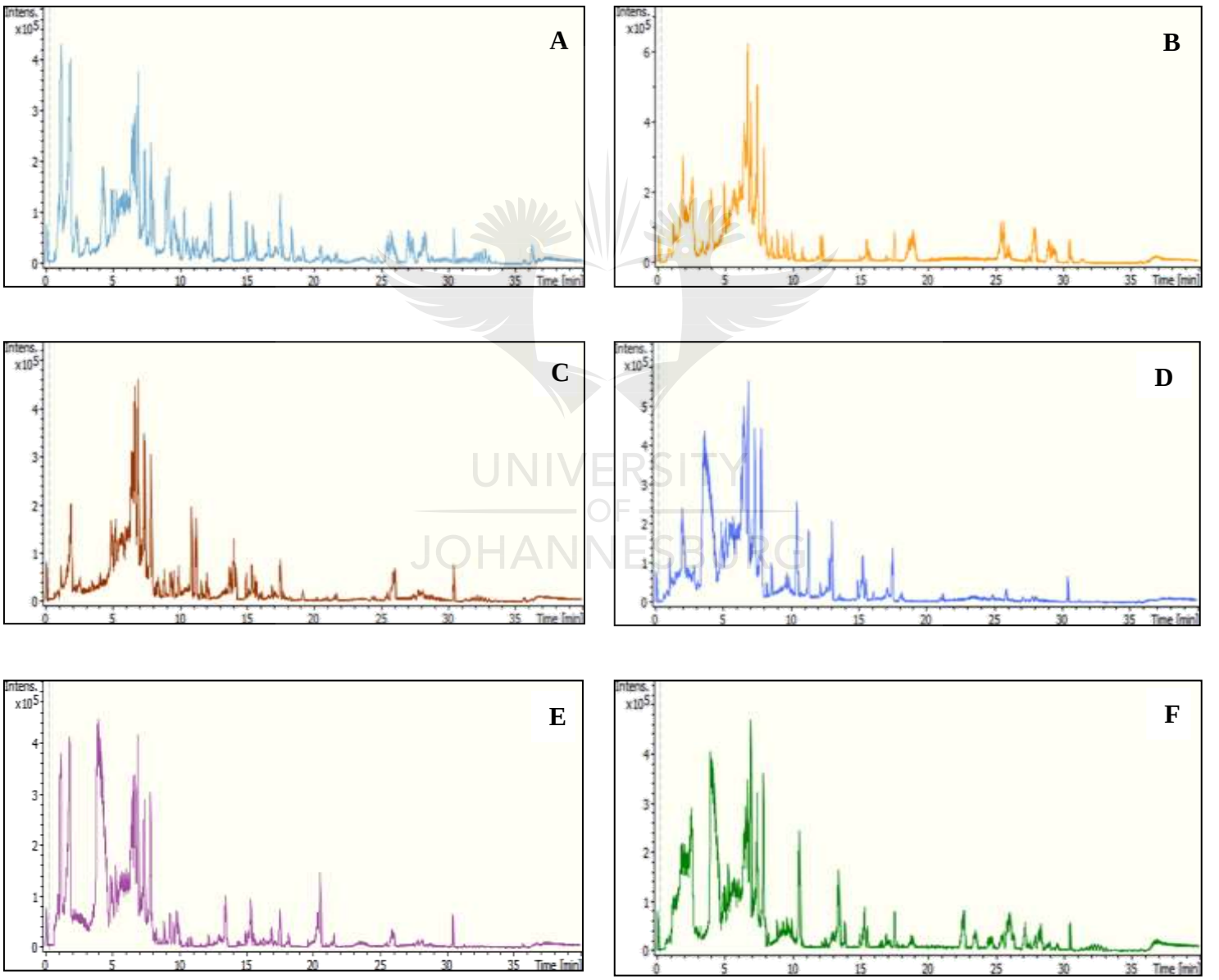
5.2.3 Data analysis

Subsequent data obtained was imported into the Bruker compass data analysis software version 4.3 (Bruker Daltonics, Bremen, Germany) for spectral data interpretation. The resulting fragment spectra were characterized using MetFrag web tool version 2.1 (https://msbi.ipb-halle.de/MetFragBeta/) linked to three different databases: KEGG, PubChem, ChemSpider. The following settings were applied in the MetFrag search: precursor ion = $[M + H]^+$; charge = positive and mode = $[M+ H]^+$; database search relative mass deviation (search ppm) = 10.0; fragment peak match absolute mass deviation (Mzabs) = 0.01; fragment peak match relative mass deviation (Mzppm) = 10.

5.3 RESULTS AND DISCUSSIONS

5.3.1 Secondary metabolites from endophytic bacteria

Representative chromatograms of crude extracts from the different endophytic bacteria isolated from *S. mauritianum* is shown in Figure 5.1 A – G. There were observed similarities, but also differences in the chromatograms. Subsequent metabolites identified from these peaks across all tested bacterial endophytic samples are presented in Tables 5.1). Most of the compounds were found reoccurring among the identified endophytes as well as endophytes of same family/genus.



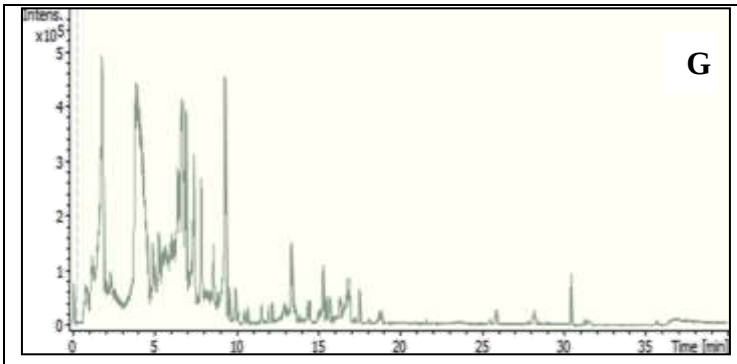


Figure 5.1: Base peak chromatograms (BPCs) of the crude extract from the different endophytic bacteria isolated from S. mauritianum.

Note: A = *P. ananatis*, B = *Xanthomonas* sp., C = *P. eucalypti*, D = *B. safensis*, E = *P. vagans*, F = *B. licheniformis*, G = *Arthrobacter* sp.

Table 5.1: Identification of secondary metabolites in bacterial endophytes isolated from *S. mauritianum*

Rt (min)	$[M + H]^+$ (*m/z*)	MS^2 (*m/z*)	Err (ppm)	Metabolite name	Type of compound	Samples
2.81	276	91, 120, 175, 231, 259, 276	-9.8	Dubinidine	Quinoline alkaloid	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As*
2.80	227	86, 114, 153, 181, 211, 227	1.8	Genipin	Monoterpenoid (Iridoid)	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As*
2.89	285	82, 83, 110, 166, 257, 285	-9.5	Xanthosine	Purine alkaloid	*Pa*, *Xs*, *Pe*, *Pv*, *Bl*, *As.*
3.15	205	91, 115, 146, 159, 188, 205	-13.2	Droserone	α-Naphthoquinone	*Pa*, *Xs*, *Pe*, *Pv*, *Bl*, *As.*
4.16	144	77, 89, 115, 127, 128, 144	-17.4	4-Methyl-5-(2'-hydroxyethyl)-thiazole	Thiazole alkaloid	*Pa*, *Bs*, *Pv*, *Bl*, *As.*
4.23	161	77, 91, 115, 144, 155, 161	-16.8	Anatabine	Pyridine alkaloid	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As.*
5.14	301	130, 284, 301	2.0	Kaempferide	Flavonoid (Flavonol)	*Pe*
5.74	231	74, 91, 158, 183, 214, 231	6.1	Visnagin	Pyrone (Chromone)	*Xs*, *Bs*, *Pv*, *Bl*, *As.*
5.99	261	86, 120, 136, 155, 215, 261	-10.3	Khellin	Pyrone (Chromone)	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As.*
6.58	247	70, 120, 174, 219, 246, 247	-15.8	(R)-Columbianetin	Furanocoumarin	*Pa*, *Xs*, *Pe*, *Bs*, *Bl*, *As*
6.80	183	89, 115, 140, 141, 168, 183	0.5	1-Methyluric acid	Purine alkaloid	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *As.*
6.94	211	70, 86, 138, 154, 183, 211	6.6	Sinapyl alcohol	Monolignol	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As*
6.95	421	70, 114, 183, 211, 421	-6.4	Eupaformosanin	Sesquiterpenoid	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As.*
7.14	174	86, 100, 144, 174	-16.1	Swainsonine	Indolizidine alkaloid	*Xs*, *Bs*
7.44	245	70, 120, 154, 172, 217, 245	-14.3	Fulvoplumierin	Monoterpenoid	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As*
7.57	371	86, 110, 197, 285, 371	-7.3	Sophoraisoflavanone A	Isoflavanone	*Pv*, *As*
8.18	261	86, 98, 120, 130, 233, 261	-10.3	8-Deoxylactucin	Sesquiterpenoid (Guaianolide)	*Bs*, *Pv*
8.52	427	86, 159, 268, 427	-6.3	Archangelicin	Coumarin (Furanocoumarins)	*Pe*
8.68	286	103, 130, 285, 286	-9.4	Arborinine	Acridone alkaloid	*Pe*, *Pv*, *Bl*
8.78	234	85, 104, 133, 150, 186, 234	-11.5	Casimiroin	Quinoline alkaloid	*Pe*, *As*
9.16	316	86, 130, 243, 270, 298, 316	-8.5	Rosinidin	Flavonoid (Anthocyanin)	*Xs*, *Bs*, *Pv*, *Bl*, *As*
9.31	203	91, 115, 117, 144, 160, 203	-13.2	Vasicinone	Quinazoline alkaloid	*Bs*, *Bl*, *As*
9.33	405	144, 203, 405	-0.2	Mallotophenone	NS	*As*

Rt (min)	$[M + H]^+$ (*m/z*)	MS^2 (*m/z*)	Err (ppm)	Metabolite name	Type of compound	Samples
9.55	609	70, 183, 351, 478, 609	-4.4	Reserpine	Indole alkaloids	*Bs*
10.40	344	86, 199, 227, 231, 281, 344	-7.8	(-)-Laudanidine	Isoquinoline alkaloid	*Pa*, *Xs*, *Bs*, *Pv*, *Bl*
10.59	391	72, 159, 391	-6.9	Loganin	Monoterpenoid (Iridoid)	*Pe*
10.66	395	159, 250, 395	-5.6	Deguelin	Isoflavonoid (Rotenone)	*Pe*
11.69	289	99, 130, 185, 271, 273, 289	-1.4	Asebogenin	Flavonoid (Dihydrochalcone)	*Pa*, *Xs*, *Pe*, *Bs*, *Pv*, *Bl*, *As*
11.99	435	72, 186, 250, 435	-6.2	Graminiliatrin	Sesquiterpenoid (Guaianolide)	*Pe*
13.42	223	65, 93, 121, 148, 223	-12.1	Leptodactylone	Coumarin	*Xs*
13.70	451	159, 215, 276, 433, 451	8.2	Auriculoside	Flavonoid glycoside	*Pe*
13.77	287	94, 120, 141, 185, 241, 287	-9.4	Kaempferol	Flavonoid (Flavonol)	*Xs*
13.79	360	216, 243, 360	-7.5	Triglochinin	Cyanogenic glycoside	*Pe*, *Bs*
13.98	288	86, 121, 132, 185, 270, 288	-1.4	Mesembrenone	Isoquinoline alkaloid	*Xs*, *Pe*
14.24	284	95, 284	3.9	Acrophylline	Quinoline alkaloid	*Pe*
16.79	415	119, 415	-7.9	Picropodophyllin	Lignan	*Pa*, *Pe*, *Bs*, *Pv*, *Bl*, *As*
16.87	273	158, 217, 245, 273	-9.9	Naringenin	Flavonoid (Flavanone)	*Pa*
17.10	330	81, 121, 312, 330	-8.2	Hetisine	Terpenoid alkaloid	*Xs*
17.29	288	74, 92, 106, 185, 270, 288	-0.7	Isolobinine	Piperidine alkaloid	*Pe*, *Bs*, *Bl*
17.82	359	99, 271, 341, 359	-7.5	Columbin	Diterpenoid (Clerodane)	*Xs*
21.48	279	93, 121, 148, 278, 279	-9.7	Tuliposide A	NS	*Pe*, *Bs*, *Pv*, *Bl*, *As*
21.95	625	107, 275, 445, 625	-0.9	Dauricine	Isoquinoline alkaloid	*Pe*, *Bl*
22.11	406	136, 182, 406	-2.7	Icacine	Terpenoid alkaloid	*Xs*
22.20	241	69, 83, 95, 97, 149, 241	-15.3	Borrerine	Indole alkaloid	*Pa*, *Xs*, *Pv*
25.09	353	84, 129, 353	-7.6	Montanol	Diterpenoid	*Xs*
25.87	331	85, 95, 239, 313, 331	-8.2	Cannabielsoin	Polyketide (Cannabinoid)	*Pe*, *Bs*, *Pv*, *Bl*, *As*
27.77	298	85, 242, 257, 298	-9.1	Cassine	Piperidine alkaloid	*Xs*, *Bl*

Rt (min)	$[M + H]^+$ (*m/z*)	MS^2 (*m/z*)	Err (ppm)	Metabolite name	Type of compound	Samples
29.44	1049	86, 441, 595, 685, 699, 1049	7.5	Protodioscin	Terpenoid (Steroidal saponin)	*Pa*, *Bl*, *As*
30.62	391	71, 148, 391	1.8	Neoquassin	Triterpenoid	*Bs*, *Pv*, *Bl*, *As*

Rt = Retention time in minutes. The RT reported here is the average retention time of same compound identified across the samples; *m/z* = mass-to-charge ratio; Err = Mass error measured in ppm; NS = Not specified; Pa = *P. ananatis*, Xs = *Xanthomonas* sp., Pe = *P. eucalypti*, Bs = *B. safensis*, Pv = *P. vagans*, Bl = *B. licheniformis*, As = *Arthrobacter* sp. Chromatograms of each compound can be found in the Appendix.

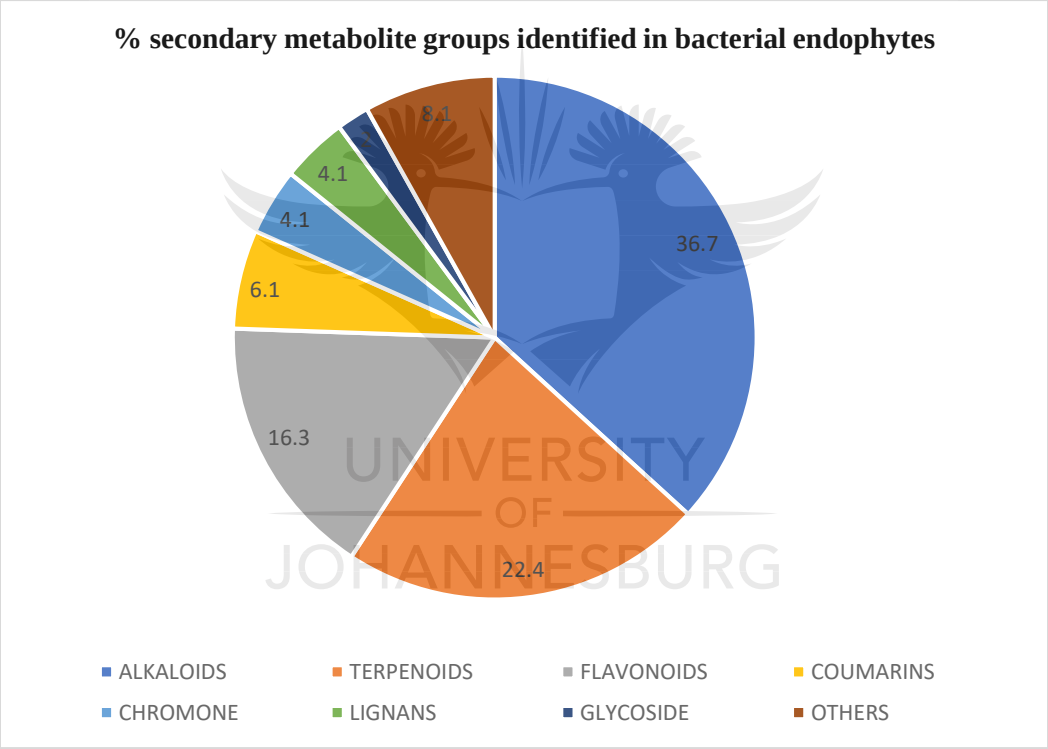


Figure 5.2: Pie chart showing the different groups of secondary metabolites identified in the bacterial endophytes isolated from S. mauritianum.

The untargeted screening of the crude endophytic extracts done in this study to the best of our knowledge is the first especially for the *Pantoea* species. Most of the compounds identified in the endophytes have not been reported present in any previous study involving other strains of the isolated endophytes used in this study. Among the compounds identified were alkaloids, flavonoids, terpenoids and a number of other groups of phytochemicals. Alkaloids, terpenoids and flavonoids were the compounds most identified in this study, constituting 36.7 %, 22.4 % and 16.3 % respectively, of the total compounds identified. The different groups of secondary metabolites were identified across all the seven isolated endophytic bacteria (*P. ananatis*, *Xanthomonas* sp., *P. eucalypti*, *B. safensis*, *P. vagans*, *B. licheniformis*, *Arthrobacter* sp.).

Alkaloids identified in bacterial endophytes

Alkaloids are grouped into several classes and they include tropanes, pyrrolidines, Isoquinoline purines, imidazoles, quinolizidines, indoles, piperidines and pyrrolizidines; most of which were identified in this study (Thawabteh *et al*., 2019). This group of phytochemicals are continuously being researched because of their impressive biological activity and medicinal uses. Alkaloids are one of the largest groups of plant secondary metabolites (Matsuura and Fett-Neto, 2015).

Dubinidine has been previously isolated from *Haplophyllum dubium* (Grundon and James, 1971) and has been reported to have CNS depressant activity (Jansen *et al*., 2006). This compound was produced by all the endophytes reported in this study. **Xanthosine** was identified in *Cynodon dactylon* by GC-MS Technique and although there is no record of it having any bioactivity, it has been reported to be a hydrogen ion symporter (Jebastella and Appavoo, 2015). **4-Methyl-5-(2'-hydroxyethyl)-thiazole** is a known metabolite of *Escherichia coli* (Mizote *et al*., 1996). To the best of our knowledge, it has no known biological activity, but alkaloids are known to have a wide range of bioactivities including antimicrobial, anti-inflammatory, antioxidant and anticancer, etc. (Jayakumar and Murugan, 2016a). **1-Methyluric acid** is one of the metabolites that was reported present in caffeine and it was reported to be particularly effective at inhibiting low-density-lipoprotein (LDL) oxidative modification (Gómez-Ruiz *et al*., 2007). **Arborinine** has been previously isolated from the leaves *Anil haplophylla* (Funayama and Cordell, 1984) the bark of *Thunbergia natalensis* (Pegel and Wright, 1960), isolated from the leaves of *Teclea trichocarpa* (Muriithi *et al*., 2002), the stem bark of *Teclea gerrardii* (Kamdem Waffo *et al*., 2007), the stems

of *Glycosmis arborea* (Ito *et al*., 2004) and from *Glycosmis parva* leaf extract (Piboonprai *et al*., 2018). Piboonprai *et al*., (2018) reported that arborinine present in *Glycosmis parva* decreased cell proliferation and was strongly cytotoxic to HeLa cervical cancer cells without significantly affecting normal cells. Arborinine was reported to have displayed antiplasmodial activity against *Plasmodium falciparum* (Dolabela *et al*., 2008). It was also reported to have demonstrated moderate *in vitro* antibacterial activity against different test bacteria *P. aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter aerogenes, Enterobacter cloacae, E. coli* and *Providencia stuartii* (Seukep *et al*., 2016).

Casimiroin is a component of the edible fruit: *Casimiroa edulis* (Maiti *et al*., 2009) and is reported to have chemopreventive potential (Ito *et al*., 1998). **Vasicinone** was present is one of the major alkaloids found in *Adhatoda vasica* (L.) Nees leaves and it is known to have bronchodilatory activity (Avula *et al*., 2008) and antiplasmodial activity (Moloudizargari *et al*., 2013). **(-)-Laudanidine** has been isolated from several species of plant including the *Papaver somniferum* L (opium poppy) the bark of *Cryptocarya amygdalina*, and several *Machilus* and *Thalictrum* species (Francis *et al*., 2008). This compound was also isolated from the roots of *Polyalthia cerasoides* and it exhibited antimalarial activity against *Plasmodium falciparum*, (Kanokmedhakul *et al*., 2007). **Mesembrenone** alkaloid is known to be a secondary metabolite of *Sceletium tortuosum* (Gericke and Viljoen, 2008; Shikanga *et al*., 2011). Mesembrenone has been found to have cytotoxic activity against a murine non-tumoral fibroblast cell line and a human tumoral cell line (Molt4) (Weniger *et al*., 1995 Gericke and Viljoen, 2008). **Acrophylline** was previously isolated from the leaves of *Acronychia haplophylla* (Epifano *et al*., 2013). Huang *et al*., (1995) reported the antiallergic activities of acrophylline.

Isolobinine is a bioactive component of *Lobelia inflata*, a medicinal plant used to treat cough (Fatoki *et al*., 2019). **Dauricine** is a constituent of *Cardiopetalum calophyllum*, *Menispermum dauricum*, and *M. dauricum* DC. (Schiff, 1997; Qian, 2002). Wang *et al*., (2012) reported that dauricine has the ability to inhibit proliferation of urinary tract tumor cells while Tang *et al*., (2009) and Yang *et al*., (2010) reported it's anticancer activities in human breast cancer and colon cancer cells respectively. **Icacine** have been reported from the leaves and roots of *Icacina guessfeldtii*, whose root decoction is used as an anticonvulsant in traditional medicine in tropical Africa (Zhao

et al., 2018). Phytochemical investigations on *Borreria verticillata* have revealed the occurrence of **Borrerine** (Ferreira Júnior *et al*., 2012). There is no available information on its pharmacological/biological activity. **Reserpine** was reported to be present in *Rauvolfia serpentine* (Negi *et al*., 2014) and has been reported to have antihypertensive properties (Varchi *et al*., 2005). The leaves of *Senna racemosa* produced the alkaloid: **Cassine**. Antimicrobial screening of the compound revealed the antibacterial activity of cassine with MIC of 2.5 mg/ml for *Staphylococcus aureus* and *Bacillus subtilis* and 5.0 mg/ml for *Candida albicans* (Sansores-Peraza *et al*., 2000) while Moo-Puc *et al*., (2007) reported its antiprotozoal activity against *Giardia intestinalis* with an IC_{50} of 3.28 µg/mL. This compound and its derivatives have also been reported to produce anti-hyperalgesics and anti-inflammatory reactions in both acute and chronic inflammatory and neuropathic pain models (Da Silva *et al*., 2012) and it also has an antiproliferative effect on HepG2 human liver cancer cell line (Pereira *et al*., 2016).

Terpenoids identified in bacterial endophytes

Terpenoids are isoprene-based natural products with fundamental roles in the metabolism of all organisms (Bergman *et al*., 2019). Terpenoids are the largest and most widespread class of secondary metabolites, mainly in plants. A few of them have been used for therapeutic purposes for centuries (las Heras *et al*., 2005). A number of terpenoids were identified in this study and they are discussed below.

The terpenoids identified in the different bacterial endophytes isolated in this study are: **Eupaformosanin**, which was previously isolated from the whole plant of *Eupatorium formosanum* and is reported to have antitumor activity (Hall *et al*., 1980). **Fulvoplumierin** is an iridoid reported present in the bark of *Plumeria rubra* and have been reported to have cytotoxic activities (Kardono *et al*., 1990). **8-Deoxylactucin** has been identified in *Lactuca sativa* L. (Seo *et al*., 2009; Michalska *et al*., 2017), *Lactuca aculeata* (Michalska and Kisiel, 2012) and *Lactuca altaica* (Beharav *et al*., 2020). It is reported to have some potential as an immunomodulator because of its influence on induction of ICAM-1 (intercellular adhesion molecule-1). It also showed significant cytotoxicity against VA-13 cells with an IC_{50} value of 8.5 µM and inhibited the growth of WI-38 cells (Zhang *et al*., 2006). **Graminiliatrin** which is present in *Liatris graminifolia* is among the sesquiterpene lactones with cytotoxic/antitumor activity as reported by Picman, (1986). **Protodioscin** is a known

steroid found in *Solanum* spp. (Abdel-Sattar *et al*., 2008; Manase *et al*., 2012), *Tribulus terrestris* (Stavrianidi *et al*., 2017), *Dioscorea* spp. (Oyama *et al*., 2017) and *Asparagus* spp. (Wang *et al*., 2003) and it has been reported as an antiproliferative compound to HL-60 Leukemic Cells (Oyama *et al*., 2017) and a potent anti-tumor agent (Cheng *et al*., 2003).

Flavonoids identified in bacterial endophytes

In addition to the high number of alkaloids identified in this study, a good number of flavonoids were also identified. Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites that have a polyphenolic structure, widely distributed in fruits, vegetables and certain beverages (Panche *et al*., 2016). Flavonoids have been reported to have very effective antioxidant, anticancer, antibacterial, anti-mutagenic, anti-inflammatory activities; as cardioprotective agents, immune system promoting ability, skin protection from UV radiation, and they interesting candidate for pharmaceutical and medical application (Kumar and Pandey, 2013; Ahmed *et al*., 2016; Panche *et al*., 2016; Tungmunnithum *et al*., 2018). A number of flavonoids were identified in the endophytic crude extracts analyzed.

In a study by Costa *et al*., (2018), five major compounds were isolated from Brazilian green propolis of which **Kaempferide** was among and was reported to have displayed gastroprotective effect. Their findings showed a diversified mode of action elicited by all compounds during the gastroprotection, including antioxidant, anti-inflammatory and antisecretory activities. Kumkarnjana *et al*., (2019) reported that Kaempferide employed its anti-adipogenic activity through inhibition of mitotic clonal expansion (MCE), either by suppressing cell proliferation or by inducing apoptosis during the early phase of differentiation. **Sophoraisoflavanone A** is reported present in *Sophora tomentosa* (Azimova and Vinogradova, 2013). The study conducted by (Sohn *et al*., 2004) reported that Sophoraisoflavanone A exhibited an impressive antifungal and antibacterial activities and an IC_{50} value of 22.1 observed when they were used on HepG2 cells. **Rosinidin** is an anthocyanin found in *Catharanthus roseus* and *Primula rosea* Royle flowers (Primulaceae) (Iwashina, 2000; Toki *et al*., 2008). There is no reported bioactivity. **Deguelin** is a natural rotenoid extracted from *Derris trifoliata* Lour. and *Mundulea sericea* (Li *et al*., 2018). It has been shown to have promising anti-cancer and anti-tumor effects against various cell lines (Suh

et al., 2013; Yu *et al*., 2017; Li *et al*., 2018; Yao *et al*., 2019) as well as cancer chemopreventive properties (Caboni *et al*., 2004).

Asebogenin is among the numerous dihydrochalcones isolated from *Piper aduncum* (Orlikova *et al*., 2011), *Lippia* species (Funari *et al*., 2012; Martins *et al*., 2019), leaves of *Pieris japonica* (Yao *et al*., 2005). It displayed very noteworthy activity towards *Bacillus subtilis* and *Micrococcus luteus* (Orjala *et al*., 1994; Rozmer and Perjési, 2016). It was also reported to have downregulated the proliferation of murine B cells (Orlikova *et al*., 2011). In another study, asebogenin was recorded as one of the most active compounds against *Cryptococcus neoformans* (strain 90012) growth, with MIC value of 15.6 μg/mL (Funari *et al*., 2012; Martins *et al*., 2019). It also has antibacterial activity (Quílez *et al*., 2010). **Auriculoside** is a compound found in *Acacia auriculiformis* (Subhan *et al*., 2018) and the roots of *Cynanchum auriculatum* (Gu and Hao, 2016) and it has been found to possess CNS depressant and antioxidant activities (Sathya and Siddhuraju, 2012; Sathya and Siddhuraju, 2013). **Kaempferol** is the most common flavonoid, which is found various in plants (for example *Cicer* spp., etc) (Azimova and Vinogradova, 2013), vegetables and fruits (Wang *et al*., 2019). Kaempferol, has anticancer, antioxidant (Li *et al*., 2015; Lee *et al*., 2014; Sekiguchi *et al*., 2019), anti-inflammatory (Tu *et al*., 2007), and anti-ulcer activities (Lee *et al*., 2014). Previous studies have shown that kaempferol can inhibit cancer cell invasion and migration of human cancer cell lines, such as those of lung cancer, breast cancer (Li *et al*., 2015; Wang *et al*., 2019), colon cancer (Lee *et al*., 2014), and leukemia (Kim *et al*., 2015). Kaempferol exerts a significant inhibitory effect on the growth of the *Helicobacter pylori* bacterium (Escandón *et al*., 2016). Studies have also shown that various flavonoids such as naringenin, kaempferol, and quercetin can inhibit or reduce the formation of *Streptococcus mutans*, *Aeromonas hydrophila*, and *Escherichia coli* O157:H7 (Winter *et al*., 1989; Lee *et al*., 2011). **Naringenin** previously identified in *Cajanus cajan* (Agus *et al*., 2017), known to possesses impressive antioxidant, anticancer, anti-inflammatory, and antimicrobial activities (Tsai *et al*., 2012; Agus *et al*., 2017; Mundlia *et al*., 2019). It has also been reported that the fungi: *S. cerevisiae* produces naringenin (Lyu *et al*., 2019).

Coumarins identified in bacterial endophytes

Coumarins are classified as a member of the benzopyrone family and are of four main types: the simple coumarins, furanocoumarins, pyranocoumarins and the pyrone-substituted coumarins (Jain

and Joshi, 2012). The coumarins are of great interest due to their biological properties which include antimicrobial, anti-tumor, anti-inflammatory activities (Jain and Joshi, 2012; Venugopala *et al*., 2013; Rohini and Srikumar, 2014).

The coumarins identified from the endophytes in this study are: (-)-Columbianetin, Archangelicin Leptodactylone. A study by Jeong *et al*., (2009) reports that **(-)-Columbianetin** extracted from *Corydalis heterocarpa* had anti-inflammatory effect and was responsible for the inhibition of inflammatory substances on activated mast cells. It has also been reported to possess analgesic, calcium channel blocking and antitumor activities (Li *et al*., 2018). **Archangelicin** is a furanocoumarin found in the roots of *Peucedanum oreoselinum* (Sarkhail, 2014), in *Angelica archangelica* Linn (Wszelaki *et al*., 2011; Kumar *et al*., 2013), in *Angelica keiskei* (Kil *et al*., 2017) and in *Archangelica officinalis* (Hawryl *et al*., 2000). It has shown positive chemopreventive activity (Kil *et al*., 2017). **Leptodactylone** is commonly found in *Leptodactylon* spp. and *Linanthus* spp. (Smith *et al*., 1982). It has also been identified in *Cyperus incompletus* Epl. (Cyperaceae) where it was reported to possess antimicrobial properties against a range of Gram-positive and Gram-negative bacteria and fungi (Dini *et al*., 1993). Yang *et al*., (2007) reported that leptodactylone isolated from *Boenninghausenia sessilicarpa* showed potent protective activity on cells infected by SARS-CoV with ratio of 60% at 100mg/ml.

Chromones identified in bacterial endophytes

Two Pyrone – chromones were identified in the endophytic crude extracts analyzed in this study: **Visnagin** and **Khellin**. Visnagin and Khellin are both found in *Ammi visnaga*. Visnagin and Khellin have biological activity as vasodilators (Lee *et al*., 2010; Sharma *et al*., 2018). Visnagin is able to reduce blood pressure by inhibiting calcium influx into the cell (Lee *et al*., 2010) and a study by Aydoğmuş-Öztürk *et al*., (2019) showed that visnagin can inhibit the proliferation of malignant melanoma by inducing the intracellular oxidative stress. Khellin on the other hand has been reported to have antispasmodic activities (Sharma *et al*., 2018). Khellin together with visnagin have been reported as bioherbicides (Travaini *et al*., 2016). Khellin and visnagin, have shown an arsenal of interesting biological activities such as antibacterial, antifungal, antiviral, antidiabetic, anti-inflammatory and neuroprotective activities (Bishr *et al*., 2018).

Lignans identified in bacterial endophytes

Another group of secondary metabolites identified in this study is Lignans. Lignans have shown interesting bioactivities which include: Anti-inflammatory, antimicrobial, antioxidant activities, antiviral, anticancer among others (Wilson *et al*., 2012). The lignans identified in endophytes are: **Sinapyl alcohol** is a lignin monomer. Lignins are natural product present in the cell walls of vascular plants (and in algae), they protect the plant against microbial attacks while giving the plant cell wall strength and rigidity (Rodrigo *et al*., 2011) and has been reported to have antinociceptive and anti-inflammatory effects (Choi *et al*., 2004). **Picropodophyllin** is a compound which occurs naturally and can be isolated from certain plant species and has been demonstrated to induce apoptosis of malignant cells as well as tumor regression in different tumor models (Ohshima-Hosoyama *et al*., 2010; Strömberg *et al*., 2015; Tarnowski *et al*., 2017).

Glycoside, steroid and other compounds identified in bacterial endophytes

Triglochinin, a cyanogenic glycoside was identified in this study. It has been previously identified in *Arum* species (Kozuharova *et al*., 2020) and there is currently no reports about its bioactivity/uses.

Another secondary metabolite identified in the endophytic bacteria is Mallotophenone. According to the report by Arisawa, (1994), **Mallotophenone**, a constituent of *Mallotus japonicus* showed significant cytotoxic activities against human epidermoid KB carcinoma cells and mouse leukemia L5178Y cells in vitro. Mallotophenone is present in *Mallotus oppositifolius* and was reported to have antiproliferative activity against the A2780 human ovarian cancer cell line with IC_{50} 6.3 ± 0.4 μM (Harinantenaina *et al*., 2013).

5.3.2 Secondary metabolites from *S. mauritianum* plant parts

Chromatograms in Figure 5.2 H - M and table (Table 5.2) summarize the secondary metabolites identified across all tested *S. mauritianum* plant parts. As expected, most of the compounds were found reoccurring among the different plant parts and a few of the compounds were also identified in the endophytes. Three major *Solanum* alkaloids were also identified in the plant.

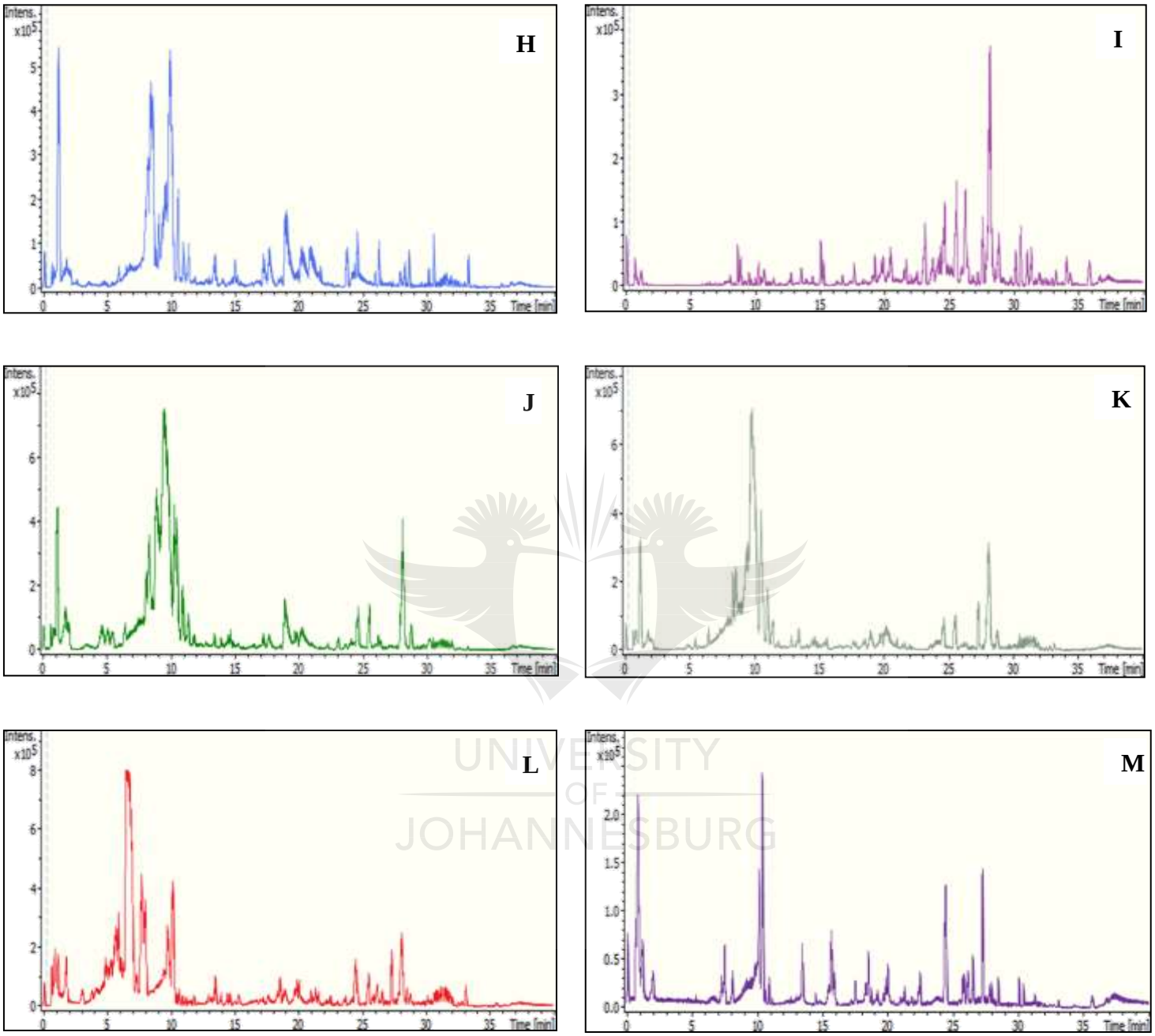


Figure 5.3: Base peak chromatograms (BPCs) of the crude extract from the different parts of S. mauritianum.

Note: **H** = Ripe fruit (coat), **I** = Ripe fruit(seeds), **J** = Green fruit (seeds), **K** = Green fruit (coat), **L** = Stem, **M** = Leaves.

Table 5.2: Identification of secondary metabolites in *S. mauritianum* plant parts.

Rt (min)	[M + H]+ (*m/z*)	MS^2 (*m/z*)	Err (ppm)	Metabolite name	Type of compound	Plant parts
1.27	276	86, 132, 161, 230, 258, 276	-12.6	Cardiospermin	Cyanogenic glycoside	RFC, GFC
1.36	266	98, 116, 230, 248, 266	-10.1	Furofoline I	Acridone alkaloid	RFC
1.55	286	80, 124, 286	-9.4	Riccionidin A	Anthocyanidin	RFC
2.13	329	120, 166, 264, 310, 329	2.7	Bergenin	C-glycoside	RFC
2.83	205	91, 115, 118, 146, 188, 205	-13.2	Droserone	α-Naphthoquinone	Stem, Leaves
3.21	308	85, 146, 230, 272, 290, 308	-8.8	Cusparine	Quinoline alkaloid	RFC
3.17	391	128, 193, 230, 307, 355, 391	-6.9	Loganin	Monoterpenoid (Iridoid)	RFC, GFS
4.99	174	144, 156, 174	-9.8	Swainsonine	Indolizidine alkaloid	Stem, Leaves
5.14	345	137, 165, 345	-7.8	Theogallin	Glycoside	RFC
5.14	303	105, 195, 285, 303	-8.9	Homoeriodictyol chalcone	Flavonoid (Chalcone)	RFC
5.72	305	70, 89, 112, 305	-8.8	Vicine	Alkaloid glycoside	GFC, Stem, Leaves
5.88	307	89, 117, 177, 248, 307	6.8	Cimifugin	Pyrone – Chromone	GFS, Stem, Leaves
5.97	304	70, 89, 112, 144, 177, 304	-8.9	Gynocardin	Cyanogenic glycoside	Stem
6.21	353	110, 136, 182, 353	-7.6	Berberastine	Isoquinoline alkaloid	RFC
6.61	193	77, 90, 118, 133, 161, 193	-4.7	Scopoletin	Coumarin	RFC, RFS, GFS, GFC
7.09	269	81, 269	-3.3	Lysergic acid	Indole alkaloid	RFC
7.28	251	79, 119, 147, 251	6.8	Glycophymoline	Quinazoline alkaloid	RFC
7.54	213	64, 135, 167, 213	-12.8	Danielone	Phytoalexin	RFS
8.39	884	70, 85, 129, 253, 414, 884	-3.1	Solasonine	Steroid alkaloid	RFC, RFS, GFS, GFC, Stem, Leaves
8.64	868	85, 129, 253, 868	-3.1	α-Solanine	Steroid alkaloid	RFC, RFS, GFS, GFC, Stem, Leaves
8.69	430	70, 157, 253, 430	-6.3	Imperialine	Steroid alkaloid	RFC, RFS, GFS, GFC, Stem, Leaves
9.06	227	68, 86, 114, 159, 227	8.8	Genipin	Monoterpenoid (Iridoid)	RFC, RFS

Rt (min)	[M + H]+ (*m/z*)	MS^2 (*m/z*)	Err (ppm)	Metabolite name	Type of compound	Plant parts
9.83	414	70, 157, 253, 414	-6.5	Solasodine	Steroid alkaloid	RFC, RFS, GFS, GFC, Stem, Leaves
10.11	576	70, 85, 157, 253, 320, 576	-4.7	α-Ergocryptine	Indole alkaloid	RFC, RFS, GFS, GFC, Stem, Leaves
10.11	869	85, 129, 253, 868, 869	-3.1	Dioscin	Terpenoid (Steroid)	GFS, Leaves
10.27	627	177, 314, 627	-4.3	Quercetin 3-O-sophoroside	Flavonoid (Flavonol)	Stem
11.12	331	158, 258, 310, 331	-8.2	Podolide	Diterpenoid	RFS
11.24	179	65, 91, 119, 147, 179	-5.0	Esculetin	Coumarin	RFS
12.57	333	95, 279, 333	5.1	Callicarpone	Diterpenoid	RFC, GFC
12.60	295	91, 93, 151,179, 277, 295	-9.1	10-Deoxysarpagine	Indole alkaloid	RFC, RFS, GFS, Leaves
16.64	353	81, 109, 163, 243, 261, 353	-7.6	Montanol	Diterpenoid	RFC, RFS, GFS, GFC, Stem, Leaves
17.26	603	171, 227, 415, 567, 603	-0.8	Glycobismine A	Acridone alkaloid	RFS
18.24	327	81, 137, 203, 249, 277, 327	-7.6	Licarin A	Lignan – Neolignan	Stem
18.37	181	77, 107, 125, 135, 163, 181	-4.4	Coniferyl alcohol	Monolignol	RFS, GFC, Stem, Leaves
18.88	519	104, 184, 281, 303, 518, 519	-6.2	Hypercalin B	Phloroglucinol	RFC, Stem
18.92	291	91, 203, 249, 273, 291	-7.9	Ribalinium	Quinoline alkaloid	RFS
19.04	329	81, 133, 187, 329	-4.6	Rhododendrin	Glycoside	RFS, Leaves
19.46	207	105, 161, 176, 207	-13.0	Eugenin	Pyrone – Chromone	RFC, RFS
19.47	697	105, 697	-3.9	Thalicarpine	Isoquinoline alkaloid	RFC
20.44	330	81, 123, 137, 279, 330	-8.2	Hetisine	Terpenoid alkaloid	RFS, Stem, Leaves
20.92	161	77, 91, 105, 116, 146, 161	-16.8	Anatabine	Pyridine alkaloid	RFC, RFS, GFC, Stem, Leaves
21.67	279	121, 148, 209, 279	5.4	Tuliposide A	NS	RFS, GFS
22.67	267	155, 209, 225, 267	3.7	Vasconine	Alkaloid	RFS
22.81	359	260, 303, 359	8.4	Columbin	Diterpenoid (Clerodane)	RFS
24.00	288	91, 196, 288	-0.3	Lycorine	Isoquinoline alkaloid	RFS

Rt (min)	[M + H]+ (*m/z*)	MS^2 (*m/z*)	Err (ppm)	Metabolite name	Type of compound	Plant parts
24.42	279	67, 81, 173, 209, 245, 279	-9.7	Lycocernuine	Quinolizidine alkaloid	GFS, GFC, Stem, Leaves
25.77	609	280, 476, 550, 609	-4.4	Diosmin	Flavonoid (Flavone)	GFC, Stem, Leaves
26.00	331	95, 239, 257, 313, 331	1.5	Cannabielsoin	Polyketide (Cannabinoid)	RFS, GFS, GFC, Stem, Leaves
26.28	281	97, 148, 221, 245, 263, 281	-14.6	Ibogamine	Indole alkaloid	RFC, GFS, GFC, Stem, Leaves
26.38	311	81, 99, 153, 243, 279, 311	-8.7	Ibogaine	Indole alkaloid	RFC
26.72	408	89, 133, 195, 391, 408	-6.6	Cassaidine	Terpenoid alkaloid	Stem
27.01	557	89, 133, 221, 436, 548, 557	-4.8	Cucurbitacin E	Triterpenoid	GFS, Stem
27.34	593	89, 460, 461, 533, 593	-5.7	Isoswertisin 2"-rhamnoside	Flavonoid (Flavone)	GFC, Stem, Leaves
27.97	497	105, 177, 184, 281, 488, 497	-11.7	Absinthin	Sesquiterpenoid (Guaianolide)	RFS, GFS, GFC, Stem
28.31	406	91, 196, 406	-0.5	Clivoline	Pyrrolizidine alkaloid	RFS
30.62	391	71, 148, 391	-6.9	Neoquassin	Triterpenoid	GFS, Leaves
31.85	421	421	-6.4	Tingenone	Triterpenoids	RFC

Rt = Retention time in minutes. The RT reported here is the average retention time of same compound identified across the samples; *m/z* = mass-to-charge ratio; Err = Mass error measured in ppm; NS = Not specified; RFC = Ripe fruit coat, RFS = Ripe fruit seed, GFS = Green fruit seed, GFC = Green fruit coat. Chromatograms of each compound can be found in the Appendix.

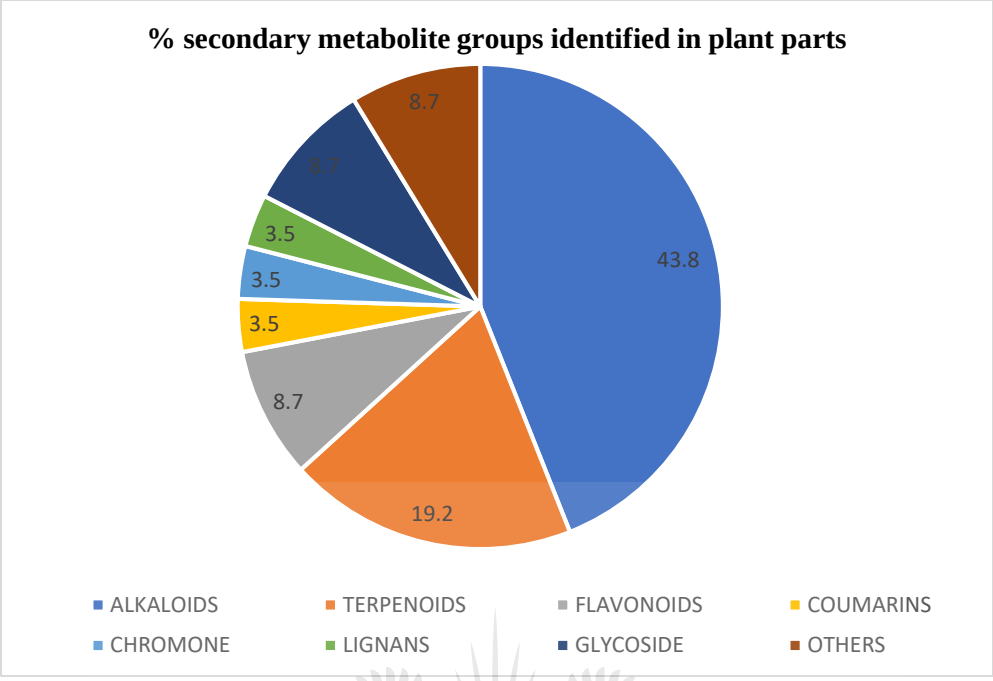


Figure 5.4: Pie chart showing the different groups of secondary metabolites identified in different parts S. mauritianum.

Alkaloids identified in *S. mauritianum* plant parts

While a good number of alkaloids were identified in the endophytes, *Solanum mauritianum* plant parts recorded a greater number of alkaloids constituting 43.8 % of the total compounds identified in the plant, a 7.1 % increase from the total number of alkaloids identified in the endophytes. *Solanum* plants are known to be rich in alkaloids (Jayakumar and Murugan, 2016b) which can also be seen with our results.

Aside the known *Solanum* alkaloids, many diverse types of alkaloids were identified. **Furofoline I** is an acridone alkaloid found in herbs and spices and have been identified in the roots of *Ruta graveolens* (rue). *In vitro* anticancer assays performed with human cell lines shows that acridone alkaloid compounds have anticancer potential (Malik *et al*., 2016). **Cusparine** is a member of quinolines and has been reported present in *Galipea officinalis* and *Angustura bark* (Sangster, 1960) and was reported by Fournet *et al*., (1993) to have potency against the parasite: *Leishmania amazonensis*. **Vicine** is one of the major metabolites found in *Vicia faba* L (faba beans) (Khazaei *et al*., 2019; Polanowska *et al*., 2019). It is known to be responsible for protection of the plant against pests and have been reported to have anti-fungal activity (Polanowska *et al*., 2019).

Berberastine was recently isolated from extracts of *Hydrastis canadensis* L (Gentry *et al*., 1998; Pillai *et al*., 2014). Berberastine has been shown to have several specific biological activities, including anti-catarrhal, anti-inflammatory, antimicrobial, laxative, emmenagogue, and oxytocic properties (Kumar and Tewari, 2018).

Two Ergot alkaloids: Lysergic acid and α-Ergocryptine were identified in this study. These types of alkaloid are particularly important as they have been reported to have the widest spectrum of biological activity reported in any family of natural products (Bur and Padwa, 2002). **Lysergic acid** has been reported to be isolated from the fungal family: Clavicipitaceae (Florea *et al*., 2017) while **α-Ergocryptine** from *Claviceps* spp. of fungi (Kantorová *et al*., 2002; Pažoutová *et al*., 2008). A study by Mulac *et al*., (2013) showed that ergot alkaloids have cytotoxic effects and induce apoptosis in two human cancer cell lines: HepG2 and HT-29 cells.

Three known *Solanum* alkaloids were identified in *S. mauritianum*: Solasonine, α-Solanine and Solasodine. **Solasonine** is a glycoalkaloid with Cholinergic activity (Jayakumar and Murugan, 2016). While glycoalkaloids are toxic compounds at certain levels, they have some positive effects. Solasonine could be used economically because its chemical structures is comparable to steroidal hormones and have been proposed to be used as an important source for the production of contraceptives and steroidal anti-inflammatory drugs (Maurya *et al*., 2009). This compound have been studied for its antidiabetic, antifungal, antiparasitic, antibiotic, antimicrobial, antiviral and mostly for anticancer properties (Lee *et al*., 2007; Li *et al*., 2007). Cancer studies on solasonine have shown significant cytotoxicity against several human cancer cell lines and skin tumors (Esteves-Souza *et al*., 2002; Maurya *et al*., 2009). **α-Solanine** is one of the numerous *Solanum* alkaloids. It has been reported to have antifungal activity, chemoprotective and chemotherapeutic effects (Komoto *et al*., 2014; Zhang *et al*., 2016; Mohsenikia *et al*., 2013; Ji and Gao, 2012), anti-inflammatory activity (Zhao *et al*., 2018). **Solasodine** is another major *Solanum* alkaloid. Several studies on this compound showed that it has antimicrobial, anti-inflammatory, antinociceptive, and anticancer activities among others (Patel *et al*., 2013; Chang *et al*., 2017; Shen *et al*., 2017; Zhuang *et al*., 2018).

Two iboga alkaloids: Ibogamine and Ibogaine were identified in *S. mauritianum***. Ibogamine** has been isolated from *Ervatamia* species, *Tabernaemontana* species, *Tabernanthe iboga* (Cardoso *et al*., 1998; Andrade *et al*., 2005; Luo *et al*., 2007; Foudjo Melacheu *et al*., 2019; Rosales *et al*., 2019). This alkaloid has shown impressive cytotoxic activity against the A2780 ovarian cancer cell line (Chaturvedula *et al*., 2003). It also decreased the self-administration of cocaine and morphine in rats (Krengel *et al*., 2019b). *Tabernaemontana* alkaloids have shown antiophidian activity antiplasmodial activity and cancer chemopreventive properties (Lavaud and Massiot, 2017; Foudjo Melacheu *et al*., 2019). **Ibogaine** is an indole alkaloid which has been extracted from the root bark of *Tabernaemontana* spp. (Kontrimavičiute *et al*., 2007; Krengel *et al*., 2019a) and has been reported to have CNS stimulant, anxiogenic, and hallucinogenic properties (Kontrimavičiute *et al*., 2007).

Imperialine has been reported present in *Fritillaria* spp. (Kiani *et al*., 2015; Yan Li *et al*., 2016; Liu *et al*., 2019; Lin *et al*., 2020). In a study conducted by Lin *et al*., (2020), they reported that Imperialine possesses anticancer and anti-inflammatory properties and Hu *et al*., (2018) reported that it contributes to the antiasthmatic effects of *Fritillaria tortifolia*. **Glycophymoline** is found in *Glycosmis arborea* and *G. pentaphylla* (da Silva *et al*., 2013). Quinazoline alkaloids are popular because of their broad range of bioactivities, including antitumor, antimalarial, antibacterial and antifungal, antiparasitic and insecticidal, antiviral, antiplatelet, anti-inflammatory, herbicidal, antioxidant activities, etc. (Shang *et al*., 2018). **10-Deoxysarpagine** has been reported in *Rauvolfia serpentina* Benth (Yu *et al*., 2002). This compound has no reported bioactivity to the best of our knowledge. **Glycobismine A** was isolated from bark and root bark *Glycosmis citrifolia* (da Silva *et al*., 2013). At a concentration of 10 mg/ml *in-vitro*, glycobismine A, suppressed *Plasmodium yoelii* (Lim, 2014). **Ribalinium** is found in the leaves of *Ruta graveolens* (Ramawat *et al*., 1985). This compound, as far as we know have not been explored with regards to its bioactivity. **Thalicarpine** is an antitumor, hypotensive, analgesic, and antimicrobial alkaloid, isolated *Thalictrum foliolosum* (Todorov and Zeller, 1992; Wu and McKown, 2002; Azimova and Yunusov, 2013; Sharma *et al*., 2020). **Vasconine** was identified in the methanolic extract *Adhatoda vasica* (Rahman *et al*., 2019) and is also an alkaloid of the *Narcissus* genus (Labrana *et al*., 2002; Bastida *et al*., 2006). There are currently not reports available on the bioactivity of this compound. **Lycorine** is an alkaloid isolated from bulbs of *Pancratium foetidum* (Bendaif *et al*.,

2018) and *Sternbergia fischeriana* (Herbert) Rupr (Saltan Çitoğlu *et al*., 2012). The anti-cancer attribute of lycorine has been characterized in human cancers and has the potential to be used in the treatment of cancers (Roy *et al*., 2018; Sun *et al*., 2018; Li *et al*., 2019). The antibacterial activity of lycorine has been evaluated. Lycorine has a moderate antibacterial activity on the majority of strains studied, nevertheless it is more effective than Streptomycin and Ampicillin against bacteria: *P. aeruginosa*, *E. cloacae* (Bendaif *et al*., 2018). Other strong pharmacodynamics properties and biological effects of this compound include hepatoprotective, antitumor, antiviral, antifungal, antimalarial, anti-inflammatory, antioxidant, antiparasitic and antifeedant (Saltan Çitoğlu *et al*., 2012). **Lycocernuine** is usually found in *Lycopodium cernuum* and other *Lycopodium* species (Morita *et al*., 2004; Morel *et al*., 2012). It has been suggested as a potential chemopreventive compound (Olafsdottir *et al*., 2013). **Cassaidine** is a terpenoid alkaloid from the genus *Erythrophleum* (Qu *et al*., 2014; Son, 2019). This alkaloid have been reported to possess inotropic action, Na+ /K+ -ATPase inhibition, and cytotoxic activity (Qu *et al*., 2014). **Clivoline** is a pyrrolizidine alkaloid. Very little is known about this compound and it has never been reported in any *Solanum* specie. Pyrrolizidine alkaloids are widely distributed worldwide in plant species relevant for human consumption. Apart from the toxicity that these molecules can cause in humans and livestock, they are also recognized for their extensive pharmacological properties which include: antimicrobial, anti-inflammatory and anti-cancer activities (Moreira *et al*., 2018).

Terpenoids identified in *S. mauritianum* plant parts

The *S. mauritianum* plant parts also recorded the presence of diverse terpenoids. They include: **Dioscin** is one of the active compounds in *Dioscoreae rhizoma*, *Dioscorea zingiberensis* and *Dioscorea nipponica* and several Dioscoreaceae plants (Tao *et al*., 2018) and it has also been isolated from *Polygonatum sibiricum* (Zhang *et al*., 2018) and *Solanum* species (Yang *et al*., 2019). Dioscin has been known for its anti-cancer activity; the study by Zhang *et al*., (2018) showed it induced the apoptosis process in HepG2 cells and Yum *et al*., (2010) reported that "real time cell analysis showed that HT-29 cells died immediately after treatment with dioscin above 7.5 μM and dioscin at 5 μM increased sub G1 phase cells when assessed with flow cytometric analysis". Dioscin can induce antioxidant enzymes, lower blood lipids, improve insulin resistance, inhibit the inflammatory response, reduce the damage of alcoholic hepatitis, and decrease the hepatic injury

induced by acetaminophen (Zhao *et al*., 2012; Kamisako and Ogawa, 2003). Furthermore, the antimicrobial, anti-inflammatory, antioxidative, and tissue-protective properties of dioscin are also widely reported (Yang *et al*., 2019). It has been found that dioscin has antitussive and antiasthmatic effects and is used to treat chronic bronchitis, and these effects are associated to its active ingredients including total saponin, water-soluble saponin, as well as steroid saponin at high quantity (Li and Du, 2018). **Podolide**, an antileukemic norditerpene dilactone, is a compound that has been reported to show tumor-inhibitory activity. This compound was responsible for the anti-tumor activity of an ethanol extract of the twigs and leaves of *Podocarpus gracilior* (Abdillahi *et al*., 2010; Abdillahi *et al*., 2012; Faiella *et al*., 2012). It also occurs in *P. falcatus* (Kupchan *et al*., 1975). Podolide showed insecticidal activity against *Heliothus zea*, *Spodoptera frugiperda* and *Pectinophora gossypiella* (Kubo *et al*., 1984). An activity-directed isolation of *P. gracilior* saw to the isolation of podolide, where it was reported to have had marginal activity in P388 lymphocytic leukemia system and was cytotoxic in 9KB nasopharyngeal carcinoma cell (Kupchan *et al*., 1975; Hembree *et al*., 1979). **Callicarpone** was reported to be present in the bark of *Cryptomeria fortune* plant (Yao *et al*., 2008) and from *Callicarpa candicans* (McChesney *et al*., 1979). To the best of our knowledge there is currently no known biological activity of this compound, but it can act as an insecticidal agent (McChesney *et al*., 1979). **Cucurbitacin E** is a compound found in plants of the Cucurbitaceae family (Habib *et al*., 2013; Chanda *et al*., 2019; Saeed *et al*., 2019), *Iberis amara* seeds (Liu *et al*., 2020), the fungus: *Curvularia lunata* (Abdelkhalek *et al*., 2017), and the medicinal plant: *Machilus yaoshansis* (Gan *et al*., 2011). Cucurbitacin E has been reported to possess anti-inflammatory (Abdelwahab *et al*., 2011; Qiao *et al*., 2013), anti-angiogenic, immunomodulatory, cytotoxic (Attard and Martinoli, 2015), anti-cancer and anti-proliferative (Gan *et al*., 2011; Habib *et al*., 2013; Habib *et al*., 2014; Abdelkhalek *et al*., 2017; Saeed *et al*., 2019), cytostatic and hepatoprotective (Arjaibi *et al*., 2017), and anti-oxidant (Habib *et al*., 2014) properties. It has been observed that the combination of cucurbitacin E with other synthetic anti-cancer drugs result in synergistic action in terms of cytotoxicity with greater efficacy in tumor growth inhibition (Sadzuka *et al*., 2008; Chanda *et al*., 2019; Liu *et al*., 2020). **Absinthin** has been isolated as a key dimeric guaianolide from *Artemisia absinthium* L. (wormwood) (Zhang *et al*., 2005; Aberham *et al*., 2010), *Artemisia caruifolia* (Ma *et al*., 2000) and also found in *Helicteres hirsuta* Lour. Stem (Pham *et al*., 2020). It has been recognized as a promising anti-inflammatory agent (Barrios, 2018). Absinthin showed inhibitory effects on SMMC-7721 cell lines *in vitro* and

therefore, they might have certain anticancer activities by damaging DNA (Liu *et al*., 2017). **Tingenone** has been previously identified in the root back of plants from the Celastraceae family (Mena-Rejón *et al*., 2007; Araujo Leon *et al*., 2015). Tingenone been shown to express antitumor, antimicrobial, insecticidal activity (Veloso *et al*., 2017).

Flavonoids identified in *S. mauritianum* plant parts

Homoeriodictyol chalcone is a member of the class of phytochemicals called chalcones that has a role as a plant metabolite. To the best of our knowledge, there is no information on the previous isolation, identification or biological activity of this compound, but Chalcones and their derivatives are generally known to demonstrate anti-inflammatory activities, and some have been indicated as antitumor agents (Xia *et al*., 2000; Santos *et al*., 2017). **Quercetin 3-O-sophoroside** is a major flavonoid in the leaves of *Poacynum hendersonii* (Li *et al*., 2017), in *Lycopersicon esculentum* Mill (Ferreres *et al*., 2010), and in *Brassica napus* L and genus *Brassica* (Shao *et al*., 2014), *Fresh Fruits of Eucalyptus maideni* (Tian *et al*., 2010). **Diosmin** (also known as: diosmetin-7-O-rutinoside) is a naturally occurring citrus flavone glycoside. It works as an antioxidant, anti-inflammatory, and analgesic agent (Fattori *et al*., 2020). It has been reported present in *Rosmarinus officinalis* leaves, flowers, stems, and roots (Del Baño *et al*., 2004) and in Lemon Juices (Caristi *et al*., 2003). This compound is used in pharmacology to alleviate venous diseases and disorders but has shown noteworthy antiproliferative activity in Caco-2 and HT-29 colon cancer cell lines with IC_{50} = 203 μM. The compound is reported to have anticancer, cardiovascular, antioxidant, and anti-inflammatory activities (Benavente-García and Castillo, 2008; Naso *et al*., 2016). **Isoswertisin 2''-rhamnoside** is a C-glycosylflavonoids found in leaves and stems of *Avena sativa* L. (Chopin *et al*., 1977; Akkol *et al*., 2011). C-glycosylflavonoids compounds are widespread in nature and have become the subject of increasing research interest due to their high biological potential (Zeng *et al*., 2013). It is believed to contribute to the wound healing and antioxidant activities of *A. sativa* (Akkol *et al*., 2011).

Coumarins identified in *S. mauritianum* plant parts

Scopoletin are coumarins that have been isolated from several plant species including plants of the Solanaceae family and particularly *Solanum* spp: *Solanum lyratum* (Gnonlonfin *et al*., 2012) and *Solanum indicum* (Syu *et al*., 2001). Scopoletin has been shown to possess antibacterial and

antifungal properties (Gnonlonfin *et al*., 2012). **Esculetin** is found in *Euphorbia lathyris*, *Artemisia capillaris*, and *Citrus limonia* (Selvasundaram *et al*., 2018). Esculetin has several pharmacological effects and multiple biochemical activities (Venugopala *et al*., 2013; Wang *et al*., 2015; Arora *et al*., 2016; Jeon *et al*., 2016) which includes: anti-cell proliferative potential in HN22 and HSC4 oral cancer cells by suppressing the activity of specificity protein 1 (SP1) (Cho *et al*., 2015); neuroprotective effect as shown by (Wang *et al*., 2012); enhancement of CCl_{4-} mediated apoptosis in rat liver (Tien *et al*., 2011); inhibiting efficacy of colon cancer cell growth through its antioxidant activity (Yum *et al*., 2015) and Chemopreventive potential (Selvasundaram *et al*., 2018).

Chromones identified in *S. mauritianum* plant parts

Two chromones were also identified in the analyzed *S. mauritianum* plant parts and this is the first report of their presence in a *Solanum* species. They are Cimifugin and Eugenin. **Cimifugin** is a bioactive component of the Chinese herb: *Saposhnikovia divaricata*, which is used in treating allergy (Wang *et al*., 2017). Wang *et al*., (2017) reports that Cimifugin suppresses allergic inflammation by reducing epithelial derived initiative key factors via regulating tight junctions. **Eugenin** is found in *Daucus carota* L. (Czepa and Hofmann, 2003) and *Pisonia aculeata* (Wu *et al*., 2011). Wu *et al*., (2011) reported that eugenin had an MIC of 103 μg/mL on *M. tuberculosis* H37Rv.

Lignans identified in *S. mauritianum* plant parts

The following lignans were identified in *S. mauritianum*: **Licarin A** is one of the bioactive neolignans found in plants such as *Myristica fragrans* Houtt. (nutmeg) (Abourashed and El-Alfy, 2016), leaves of *Magnolia ovata* (Barros *et al*., 2009), *Machilus thunbergfi* (Lee *et al*., 2004) *Aristolochia taliscana* (Matsui *et al*., 2015) and *Saururus chinensis* (Lour.) Baill (Qu *et al*., 2014). Licarin A has numerous pharmacological/biological activities: antitumor (Abourashed and El-Alfy, 2016), anticancer (Maheswari *et al*., 2018; Lee *et al*., 2004), neuroprotective effect (Choong *et al*., 2005; Qu *et al*., 2014), anti-inflammatory (Matsui *et al*., 2015), antioxidant properties (Néris *et al*., 2013; Lin *et al*., 2015), antiparasitic activity against *Schistosoma mansoni* and *Trypanosoma cruzi* (Cabral *et al*., 2010); Pereira *et al*., 2011; Meleti *et al*., 2020). **Coniferyl alcohol** is a monolignol found in *Fagopyrum esculentum* Moench (Kalinova *et al*., 2011), *Astragalus*

bungeanus (Alaniya *et al*., 2007), *Picea abies* and *Pinus sylvestris* (Hänninen *et al*., 2011), the bacteria: *Rhodococcus erythropolis* (Jaeger *et al*., 1981) and *Streptomyces* sp. NL15-2K (Nishimura, 2013). The biological activity of this compound have not been researched.

Glycosides and steroids identified in *S. mauritianum* plant parts

Cardiospermin was reported as the anxiolytic principle from ethanolic root extract of *Cardiospermum halicacabum* (Kumar *et al*., 2011). **Bergenin** is a kind of lactone mainly extracted from the Chinese cabbage plant. It has antipyretic, analgesic, antitussive, expectorant, anti-inflammatory effects (Xu *et al*., 2018), antinociceptive and anti-inflammatory (De Oliveira *et al*., 2011; Gao *et al*., 2015), antioxidant (Subramanian *et al*., 2015), hepatoprotective (Lim *et al*., 2000), and antiarthritic activity (Nazir *et al*., 2007). It has been isolated from *Bergenia* species (Siddiq *et al*., 2012; Boros *et al*., 2014), *Mallotus japonicus* (Lim *et al*., 2000) and *Peltophorum pterocarpum* flowers (Subramanian *et al*., 2015). **Theogallin** was identified in *Camellia sinensis* (tea plant) (Yao *et al*., 2004; Dai *et al*., 2020). It is a type of garlic acid predominantly found in fruits and is known to be a natural antioxidant (Arjeh *et al*., 2020). **Gynocardin** is the cyanogenetic glucoside of *Gynocardia odorata* R. Br (Conn, 1969) and has also been isolated from *Baileyoxylon lanceolatum* (Webber and Miller, 2008). **Rhododendrin** is a biologically active compound isolated from *Rhododendron* species and has been shown to exhibit analgesic/anti-inflammatory activity (Jeon *et al*., 2013; Kim *et al*., 2011). It has also been isolated from leaves of *Bergenia crassifolia* (L.) Fritsch (Shikov *et al*., 2014) and in *Cistus salviifolius* flower buds and leaves (El Euch *et al*., 2015).

Other secondary metabolites identified in *S. mauritianum* include: Riccionidin A, Danielone and Hypercalin B. The anthocyanidin **Riccionidin A** have been isolated from the cell walls of *Ricciocaros natans* (Kunz *et al*., 1994; Kunz and Becker, 1995; Philpott *et al*., 2009), in liverworts and in *Rhus javanica* (Taniguchi *et al*., 2000). No bioactivity is reported for this compound. **Danielone** is a phytoalexins. Phytoalexins are antimicrobial compounds produced by plants when they are exposed to microorganisms. Danielone has previously been isolated from *Carica papaya* fruits (Echeverri *et al*., 1997) and *Cestrum parqui* (D'Abrosca *et al*., 2004). It exhibited high antifungal activity against *Colletotrichum gloesporioides*, a pathogenic fungus of papaya (Echeverri *et al*., 1997). It is also reported to have antimicrobial and antioxidant activities (Krishna

et al., 2008; Aravind *et al*., 2013; Galang *et al*., 2016; Nafiu *et al*., 2018). **Hypercalin B** was isolated from the hexane and chloroform extracts of the aerial parts of *Hypericum acmosepalum* in a study by (Osman *et al*., 2012) where it was reported that hypercalin B exhibited antibacterial activity against *Staphylococcus aureus*, *Mycobacterium tuberculosis* H37Rv and *M. bovis* (Osman *et al*., 2012; Nogueira *et al*., 2013).

5.3.3 Secondary metabolites identified in both endophytes and *S. mauritianum* plant parts.

One of our hypotheses for this study was that the isolated endophytes will be able to produce same or similar secondary metabolites or group of secondary metabolites as their host plant. From our study, we observed that not only did the endophytes produce similar group of compounds as the host plant, they produced same secondary metabolites which were mostly alkaloids and terpenoids. A total of 11 compounds (Figure 5.5) were identified in both plant parts and different endophytes. These compounds include: Anatabine, Swainsonine, Hetisine, Genipin, Loganin, Columbin, Montanol, Neoquassin, Droserone, Cannabielsoin and Tuliposide A.

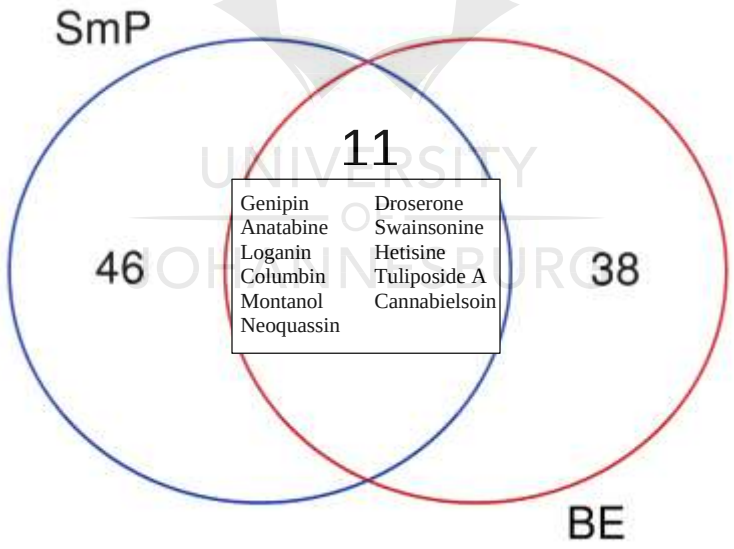


Figure 5.5: Venn diagram showing the compounds common to the bacterial endophytes analyzed in this study and S. mauritianum plant parts. Note: BE = Bacterial endophytes; SmP = S. mauritianum plant.

Anatabine is a type of alkaloid mostly found in (but not limited to) plants of the Solanaceae family and has been reported to have anti-inflammatory activities (Jenkins *et al*., 2013; Paris *et al*., 2013) and also play a role in plant defense as their bitter taste and toxicity can deter certain insect species

(Levinson, 1976). **Swainsonine** have been reported present in plant genera of Astragalus, Fabaceae, Malvaceae, Oxytropis and a host of other plants (Zhao *et al*., 2009; Cook *et al*., 2017; Cook *et al*., 2019). It has also been reported present in different fungal endophytes (Yu *et al*., 2010; Gao *et al*., 2012; Yang *et al*., 2012; Martinez *et al*., 2019; Tan *et al*., 2019). This compound is known for its anti-cancer and anti-proliferative effects and could prevent metastases (Sun *et al*., 2007; Sun *et al*., 2009; Lu *et al*., 2015; Wang *et al*., 2019). **Hetisine** is usually found in plants of the Ranunculaceae family (Grandez *et al*., 2002; Fan *et al*., 2004) and hetisine type of alkaloids are known to possess anti-inflammatory and anticancer activities (Wada *et al*., 2012; Liang *et al*., 2016). **Genipin**, has been isolated and characterized from the extract of *Gardenia jasminoides* Ellis fruit (family Rubiaceae) (Shanmugam *et al*., 2018). It has long been used in traditional oriental medicine for the prevention and treatment of several inflammation driven diseases, including cancer (Luo *et al*., 2019; Zhao *et al*., 2019). Genipin has been shown to have hepatoprotective activity acting as a potent antioxidant and inhibitor of mitochondrial uncoupling protein 2, and also reported to exert significant anticancer effects (Shanmugam *et al*., 2018).

A study on the metabolite profiling of *Parentucellia latifolia* subsp. showed that **Loganin** and its isomers were the most abundant compounds in the plant (Llorent-Martínez *et al*., 2019). Loganin has also been reported as a potential therapeutic agent for the prevention of inflammatory diseases (Yang Li *et al*., 2016). **Columbin** is found in many plants, but it was first isolated from *Tinospora bakis* (Koko *et al*., 2008; Yang *et al*., 2015). Pharmacological studies showed that this diterpenoid has multiple attractive biological activities, including anti-carcinogenesis (Zhang *et al*., 2004; Wang *et al*., 2009), anti-inflammatory (Wang *et al*., 2009; Zhang *et al*., 2004; Moody *et al*., 2006; Ibrahim Abdelwahab *et al*., 2012), and anti-hyperlipidemia (by reducing cholesterol uptake) (Nok *et al*., 2005). In vivo chemoprevention studies indicated that columbin was actively against human colon cancer carcinogenesis (Nok *et al*., 2002; Ibrahim Abdelwahab *et al*., 2012). In addition to be bioactive, columbin may also impact the metabolism of co-administered drugs, since it was shown to affect the sleeping time of anesthetized mice (Wada *et al*., 1995). **Montanol** was first isolated from the leaves of *Montanoa tomentosa* (Kanojia *et al*., 1982). To the best of our knowledge, there is no report on its bioactivity. **Neoquassin** is a Quassinoids (degraded triterpenes) reported present in members of the family Simaroubaceae (Chakraborty and Pal, 2013). The Cytotoxicity and antitumor activity, commonly found within the Simaroubaceae

family, is primarily attributed to quassinoids (Alves *et al*., 2014). **Droserone** in the pitcher plant *Nepenthes khasiana* (Nepenthaceae) was shown to have antifungal activity against a wide range of plant and human pathogens such as *Candida* and *Aspergillus* (Eilenberg *et al*., 2010). Devi *et al*., (2016) reported that droserone inhibited plant pathogens such as *Botrytis cineria*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Mycosphaerella graminicola* and also inhibited the growth of human fungal pathogens such as *Candida albicans* CBS 562, *C. albican* mas, *C. krusei*, *C. glabrata*, *Aspergillus flavus*, *A. niger*, *A. fumigates* indicating that the compound is generally an antifungal agent. Droserone was also found in the extract of *Ancistrocladus abbreviatus* and the cell cultures of *Triphyophyllum peltatum* (Bringmann *et al*., 2000; Bringmann *et al*., 2008). **Cannabielsoin** is a Cannabinoid compound found in *Cannabis sativa* L (Delgado-Povedano *et al*., 2019) and recent studies indicate that cannabinoids has shown potential as an antipsychotic (Li *et al*., 2020). **Tuliposide A** is found in *Tulipa gesneriana* L.and is a major fungitoxic agent (Hutchinson, 1974; Lubbe *et al*., 2013).

In total, 106 secondary metabolites belonging to diverse phytochemical groups were identified in both crude extracts of plant and bacterial endophytes and as seen in figure 5.2, 40.6 % of the total identified compounds in both plants and endophytes were alkaloids.

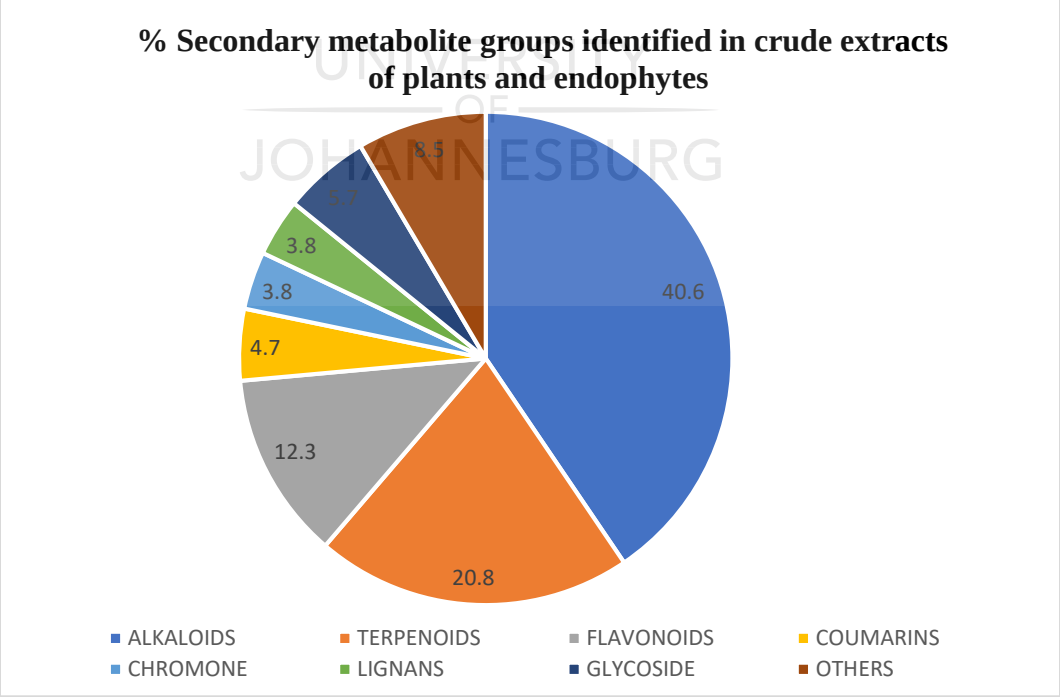


Figure 5.6: Pie chart showing the statistics of total number of secondary metabolites identified in the bacterial endophytes and aerial parts of Solanum mauritianum.

Seeing that there is limited information on the secondary metabolites present in bacterial endophytes this screening was necessary as it points to the possible active components of the endophyte which could later be isolated for therapeutic purposes. This study also shows that endophytes from *S. mauritianum* contained similar secondary metabolites as their host plant. The endophytes contained more alkaloids than any other group of secondary metabolite which could be due to the high content of alkaloid typically seen of *Solanum* species. (Jayakumar and Murugan, 2016b). This supports the report of endophytes producing similar compounds as their host plant due to genetic recombination (Tan and Zou, 2001).

Crude extracts may contain a high number of individual constituents at varying concentrations and studies have shown that the general activity of crude extracts can come from mixtures of compounds with synergistic, antagonistic or additive activity (Wagner and Ulrich-Merzenich, 2009; Junio *et al*., 2011; Caesar and Cech, 2019). More attention is given to the synergistic and additive effects, but antagonism is often overlooked. Antagonism, a phenomenon in which effects of active constituents are masked by other compounds in a composite mixture, also happens in natural product mixtures. This could be seen in our anticancer results. While the extracts worked well on bacteria, the extracts were inactive against cancer cells despite the presence of several anticancer compounds as seen in the LC-QTOF-MS/MS results.

5.4 CONCLUSION

The results of this analysis show that *S. mauritianum* and its bacterial endophytes synthesize very important biologically active compounds that can be employed in the fight against microbial drug resistance. The compounds identified to be present in the crude extracts show that microbes could be explored for their natural products to reduce the rate at which plants are harvested for natural product studies and help maintain and preserve biodiversity. The compounds reported to have no record of biological activities can be further studied to ascertain their pharmacological prospects. The extracts from this plant can be further explored for the isolation of the bioactive compounds present for possible synthesis of new pharmacological products for the treatment of ailments and also address the resistance of current drugs by microbes. Seeing as most of the identified compounds, for example: swainsonine, neoquassin, cassine, protodioscin and the *Solanum* alkaloids (Solasonine, α-Solanine and Solasodine) are said to have anticancer activities and our

anticancer experiment showed that the crude extracts in this study are not active against the tested cancer cells, it becomes necessary to clarify the synergistic and antagonistic effect of these compounds and their pharmacological mechanisms.

Lastly, this untargeted analysis validated the hypotheses of this study: First, *S. mauritianum* is a plant that is rich in bioactive phytochemicals and can be a source for possible drug leads to address the issue of antibacterial resistance, third, bacterial endophytes from *S. mauritianum* produce similar bioactive secondary metabolites as the plant. and fourth, if plants and their resident microbes are able to produce similar secondary metabolites, the use of plants in drug discovery can be substituted with the use of their microbial symbionts to avoid over harvesting plants and over exploiting our biodiversity.

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CHAPTER SIX

6.0 GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK.

6.1 GENERAL DISCUSSIONS AND CONCLUSION

Bioprospecting of natural products is a route for the discovery of sources of new drugs via the isolation of bioactive metabolites from living organisms (Buatong *et al*., 2011; Alvin *et al*., 2014; Martinez-Klimova *et al*., 2017). The microorganisms residing within plants are increasingly becoming the object of research efforts (Ambrose et al., 2013; Martinez-Klimova *et al*., 2017), especially when the source plant is used in traditional medicine. More and more information is becoming available in literature on the biodiversity and biosynthetic potential of endophytes (Martinez-Klimova *et al*., 2017). Recent studies continue to demonstrate the boundless ability of endophytes to produce biologically active compounds.

Endophytes have evolved mechanisms that allow them to compete with other microorganisms for the microhabitat inside plants. Therefore, endophytic microorganisms are a good place to search for antimicrobial and antifungal agents. Endophytes have been studied for their ability to produce antibacterial, antiviral, anticancer, antioxidant, antidiabetic and immunosuppressive compounds. This study demonstrates that metabolites with activity against bacteria, fungi and protozoa can be obtained from endophytic microorganisms. Compounds such as alkaloids, flavonoids, lignans, coumarins are examples of different metabolites that were found in the crude extracts of the bacterial endophytes analyzed in this study.

A potential advantage of researching endophytes, as suggested by Verma *et al*. (2009) is that new microbial lifestyles might increase the likelihood of finding novel bioactive compounds. Endophytes that produce antimicrobial agents may be implemented also in agriculture as an environmentally-friendly, self-propagating pest control. Scientific discoveries contribute to enhance the value of biodiversity: new bioactive drugs and new microorganisms are waiting to be discovered. Important ethnobotanical medicinal knowledge is directly linked to the success of finding bioactive molecules as demonstrated by Castillo *et al*. (2002) and Alvin *et al*. (2016), when

ethnobotanical knowledge is taken seriously at the time of selecting a medicinal plant to collect, the reward frequently is the isolation of endophytes that produce bioactive compounds.

The contributions of this study to the body of science are outlined below:

1. The surface sterilized plant parts of *Solanum mauritianum* harvested in the Doornfontein area of Johannesburg yielded 7 bacterial endophytes. These endophytes were of different species; some were from the same genus. They indicated that plants do indeed harbor a variety of microbial species.
2. The isolated bacterial endophytes were sequenced and characterized genetically based on their 16S rRNA genes. The results were as follows: three *Pantoea* species: *P. ananatis* (NU 01), *P. eucalypti* (NU 03) and *P. vagans* (NU 05); two *Bacillus* species: *B. safensis* (NU 04) and *B. licheniformis* (NU 06); one *Arthrobacter* sp. (NU 07) and one *Xanthomonas* sp. (NU 02). The gene sequences were deposited in GenBank and accession numbers were assigned to each isolate (MK070326, MK070328, MK070330, MK070329, MK070331, MK554845 and MK070327 respectively).
3. Crude extracts of isolated endophytes had noteworthy antibacterial activity against pathogenic test organisms like *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Mycobacterium smegmatis, Mycobacterium marinum, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus epidermidis.* These organisms are known to cause various infections like skin infections, foodborne infections, respiratory tract infections (e.g. tuberculosis), nosocomial infections, etc. The crude extracts were observed to be more active against the Gram-positive organisms than the Gram-negative organisms. The endophytes did not exhibit cytotoxic effects against Glioblastoma cells and Lung carcinoma cells. Therefore, these endophytes could be potential source of antibacterial compounds.
4. The plant parts antimicrobial activities and their effects on the cancer cells were very similar to those of the endophytic crude samples; while they had antibacterial effects against the test organisms, they showed no anticancer effects on the test cancer cells. The plant part (ripe fruit cover) that showed 37 % in cell viability was the most active sample. Crude extracts are generally considered to have cytotoxic activity if they reduce cell viability by at least 50 %.

5. Untargeted screening of the crude extracts of both endophytes and plant for the presence of bioactive secondary metabolites revealed the presence of 106 compounds including alkaloids, flavonoids, lignans, coumarins, terpenoids, etc. Of all these compounds, the crude extracts had more alkaloids (40.6 %) than any other class of phytochemical (see figure 5.2 in Chapter 5). Considering that *Solanum* spp. are known to be rich in alkaloids and endophytes usually synthesize similar compounds as their host plants, it is not surprising that alkaloids were the most abundant group of phytochemicals in both the plant and its bacterial endophytes.
6. While the plant parts and bacterial endophytes had their individual compounds, some compounds were observed in both the plants and endophytes. The compounds are as follows: anatabine, swainsonine, neoquassin, genipin, droserone, loganin, hetisine, columbin, tuliposide A, montanol and cannabielsoin. This justified reports that endophytes produce similar compounds as the plant(s) they are in symbiotic relationship with.

In conclusion, it is established from this study that *Solanum mauritianum*, a plant used in South African traditional medicine is a plant that hosts diverse endophytic bacterial species in its aerial parts. The crude *S. mauritianum* plant parts and their endophytic bacterial crude secondary metabolite extracts had impressive antibacterial activities against common pathogenic bacteria. They were not significantly active against Glioblastoma cells and Lung carcinoma cancer cells. These crude extracts showed the presence of a wide range of biologically active secondary metabolites. The results from this study supported all the hypothesis of this study and opens up an avenue for the isolation and characterization of new biologically active compounds with possible pharmacological use.

6.2 RECOMMENDATIONS FOR FUTURE WORK

Based on the results of this study, the following recommendations can be taken into consideration:

1. The microorganisms can be grown on a larger scale for the production of more secondary metabolites with different media that cater for the growth requirements of each organism. This could help them produce more secondary metabolites and effectively turn more natural product research to the use of microbes instead of over harvesting of plants.

2. The synergistic effect of crude extracts could be studied to better understand why the crude extracts were inactive against cancer cells while the secondary metabolite screening showed the presence of several anticancer compounds in the extracts.
3. The genes responsible for the production of secondary metabolites could be identified. Enhancing the expression of genes involved in secondary metabolite synthesis could lead to improvement in the production of secondary metabolites.
4. Since it has been established that plants and endophytes produce similar compounds, natural product researchers could consider microbes as their new source of bioactive compounds and help preserve biodiversity.

Endophytes and microorganisms at large promises to be very useful in drug development research and should be given more attention.

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APPENDICES

APPENDIX I

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MDPI

Article

Antibacterial Activities of Crude Secondary Metabolite Extracts from *Pantoea* Species Obtained from the Stem of *Solanum mauritianum* and Their Effects on Two Cancer Cell Lines

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Abstract: Endophytes are microorganisms that are perceived as non-pathogenic symbionts found inside plants since they cause no symptoms of disease on the host plant. Soil conditions and geography among other factors contribute to the type(s) of endophytes isolated from plants. Our research interest is the antibacterial activity of secondary metabolite crude extracts from the medicinal plant *Solanum mauritianum* and its bacterial endophytes. Fresh, healthy stems of *S. mauritianum* were collected, washed, surface sterilized, macerated in PBS, inoculated in the nutrient agar plates, and incubated for 5 days at 30 °C. Amplification and sequencing of the *16S rRNA* gene was applied to identify the isolated bacterial endophytes. These endophytes were then grown in nutrient broth for 7–14 days, after which sterilized Amberlite® XAD7HP 20–60 mesh (Merck KGaA, Darmstadt, Germany) resin was added to each culture to adsorb the secondary metabolites, which were later extracted using ethyl acetate. Concentrated crude extracts from each bacterial endophyte were tested for antibacterial activity against 11 pathogenic bacteria and two human cancer cell lines. In this study, a total of three bacterial endophytes of the *Pantoea* genus were identified from the stem of *S. mauritianum*. The antibacterial test showed that crude secondary metabolites of the endophytes and stem of *S. mauritianum* possessed antibacterial properties against pathogenic microbes such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, with concentrations showing inhibition ranging from 0.0625 to 8.0000 mg/mL. The anticancer analysis showed an increase in cell proliferation when A549 lung carcinoma and UMG87 glioblastoma cell lines were treated with both the plant and endophytes' crude extracts. As far as we know, this is the first study of its kind on *Solanum mauritianum* in South Africa showing *S. mauritianum* endophytes having activity against some of the common human pathogenic organisms.

Keywords: antibacterial; anticancer; bacterial endophytes; MIC plants; *Solanum mauritianum*; *16S rRNA*

APPENDIX II

MOLECULAR IDENTIFICATION OF ISOLATED ENDOPHYTES.

The following are the accession numbers and gene sequences of identified bacterial endophytes from *Solanum mauritianum* plant reported in this study.

MK070326 – *Pantoea ananatis* NU 01

CGGGTGAGTA ATGTCTGGGG ATCTGCCCGA CAGAGGGGGA TAACCACTGG AAACGGTGGC TAATACCGCA TAACCTCGCA AGAGCAAAGA GGGGGACCTT CGGGCCTCTC GCTGTCGGAT GAACCCAGAT GGGATTAGCT AGTAGGTGGG GTAACGGCTC ACCTAGGCGA CGATCCCTAG CTGGTCTGAG AGGATGACCA GCCACACTGG AACTGAGACA CGGTCCAGAC TCCTACGGGA GGCAGCAGTG GGGAATATTG CACAATGGGC GCAAGCCTGA TGCAGCCATG CCGCGTGTAT GAAGAAGGCC TTCGGGTTGT AAAGTACTTT CAGCGGGGAG GAAGGCGATG CGGTTAATAA CCGCGTCGAT TGACGTTACC CGCAGAAGAA GCACCGGCTA ACTCCGTGCC AGCAGCCGCG GTAATACGGA GGGTGCAAGC GTTAATCGGA ATTACTGGGC GTAAAGCGCA CGCAGGCGGT CTGTCAAGTC AGATGTGAAA TCCCCGGGCT TAACCTGGGA ACTGCATTTG AAACTGGCAG GCTAGAGTCT TGTAGAGGGG GGTAGAATTC CAGGTGTAGC GGTGAAATGC GTAGAGATCT GGAGGAATAC CGGTGGCGAA GGCGGCCCCC TGGACAAAGA CTGACGCTCA GGTGCGAAAG CGTGGGGAGC AAACAGGATT AGATACCCTG GTAGTCCACG CCGTAAACGA TGTCGACTTG GAGGCTGTTC CCTTGAGGAG TGGCTTCCGG AGCTAACGCG TT

MK070327 – *Xanthomonas* sp. NU 02

ACGGGTGAGT AATGCATCGG GACCTACCCA GACGTGGGGG ATAACGTAGG GAAACTTACG CTAATACCGC ATACGTCCTA CGGGAGAAAG CGGGGGATCG CAAGACCTCG CGCGGTTGGA TGGACCGATG TGCGATTAGC TAGTTGGTAA GGTAATGGCT TACCAAGGCG ACGATCGCTA GCTGGTCTGA GAGGATGATC AGCCACACTG GGACTGAGAC ACGGCCCAGA CTCCTACGGG AGGCAGCAGT GGGGAATATT GGACAATGGG CGCAAGCCTG ATCCAGCAAT GCCGCGTGTG TGAAGAAGGC CCTCGGGTTG TAAAGCACTT TTATCAGGAG CGAAATCTGC ATGGTTAATA CCCGTGTAGT CTGACGGTAC CTGAGGAATA AGCACCGGCT AACTCCGTGC CAGCAGCCGC GGTAATACGG AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGT GCGTAGGCGG TTCGTTAAGT CTGCTGTGAA AGCCCCGGGC TCAACCTGGG AATGGCAGTG GATACTGGCG AGCTAGAGTG TGTCAGAGGA TGGTGGAATT CCCGGTGTAG CGGTGAAATG CGTAGAGATC

GGGAGGAACA TCAGTGGCGA AGGCGGCCAT CTGGGACAAC ACTGACGCTG AGGCACGAAA GCGTGGGGAG CAAACAGGAT TAGATACCCT GGTAGTCCAC GCCCTAAACG ATGCGAACTG GATGTTGGTC TCAACTCGGA GATCAGTGTC GAAGCTAACG CGTT

MK070328 – *Pantoea eucalypti* NU 03

TGGCGGACGG GTGAGTAATG TCTGGGGATC TGCCCGATAG AGGGGGATAA CCACTGGAAA CGGTGGCTAA TACCGCATAA CGTCGCAAGA CCAAAGAGGG GGACCTTCGG GCCTCTCACT ATCGGATGAA CCCAGATGGG ATTAGCTAGT AGGCGGGGTA ATGGCCCACC TAGGCGACGA TCCCTAGCTG GTCTGAGAGG ATGACCAGCC ACACTGGAAC TGAGACACGG TCCAGACTCC TACGGGAGGC AGCAGTGGGG AATATTGCAC AATGGGCGCA AGCCTGATGC AGCCATGCCG CGTGTATGAA GAAGGCCTTC GGGTTGTAAA GTACTTTCAG CGGGGAGGAA GGCGATRCGG TTAATAACCG CGTCGATTGA CGTTACCCGC AGAAGAAGCA CCGGCTAACT CCGTGCCAGC AGCCGCGGTA ATACGGAGGG TGCAAGCGTT AATCGGAATT ACTGGGCGTA AAGCGCACGC AGGCGGTCTG TTAAGTCAGA TGTGAAATCC CCGGGCTTAA CCTGGGAACT GCATTTGAAA CTGGCAGGCT TGAGTCTTGT AGAGGGGGGT AGAATTCCAG GTGTAGCGGT GAAATGCGTA GAGATCTGGA GGAATACCGG TGGCGAAGGC GGCCCCCTGG ACAAAGACTG ACGCTCAGGT GCGAAAGCGT GGGGAGCAAA CAGGATTAGA TACCCTGGTA GTCCACGCCG TAAACGATGT CGACTTGGAG GTTGTTCCCT TGAGGAGTGG CTTCCGGAGC TAACGCGTT

MK070329 – *Bacillus safensis* NU 04

GACGGGTGAG TAACACGTGG GTAACCTGCC TGTAAGACTG GGATAACTCC GGGAAACCGG AGCTAATACC GGATAGTTCC TTGAACCGCA TGGTTCAAGG ATGAAAGACG GTTTCGGCTG TCACTTACAG ATGGACCCGC GGCGCATTAG CTAGTTGGTG GGGTAATGGC TCACCAAGGC GACGATGCGT AGCCGACCTG AGAGGGTGAT CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG TAGGGAATCT TCCGCAATGG ACGAAAGTCT GACGGAGCAA CGCCGCGTGA GTGATGAAGG TTTTCGGATC GTAAAGCTCT GTTGTTAGGG AAGAACAAGT GCGAGAGTAA CTGCTCGCAC CTTGACGGTA CCTAACCAGA AAGCCACGGC TAACTACGTG CCAGCAGCCG CGGTAATACG TAGGTGGCAA GCGTTGTCCG GAATTATTGG GCGTAAAGGG CTCGCAGGCG GTTTCTTAAG TCTGATGTGA AAGCCCCCGG CTCAACCGGG GAGGGTCATT GGAAACTGGG AAACTTGAGT GCAGAAGAGG AGAGTGGAAT TCCACGTGTA GCGGTGAAAT GCGTAGAGAT GTGGAGGAAC ACCAGTGGCG AAGGCGACTC TCTGGTCTGT AACTGACGCT GAGGAGCGAA AGCGTGGGGA GCGAACAGGA TTAGATACCC

TGGTAGTCCA CGCCGTAAAC GATGAGTGCT AAGTGTTAGG GGGTTTCCGC CCCTTAGTGC TGCAGCTAAC GC

MK070330 – *Pantoea vagans* NU 05

AGTGGCGGAC GGGTGAGTAA TGTCTGGGGA TCTGCCCGAT AGAGGGGGAT AACCACTGGA AACGGTGGCT AATACCGCAT AACGTCGCAA GACCAAAGAG GGGGACCTTC GGGCCTCTCA CTATCGGATG AACCCAGATG GGATTAGCTA GTAGGCGGGG TAATGGCCCA CCTAGGCGAC GATCCCTAGC TGGTCTGAGA GGATGACCAG CCACACTGGA ACTGAGACAC GGTCCAGACT CCTACGGGAG GCAGCAGTGG GGAATATTGC ACAATGGGCG CAAGCCTGAT GCAGCCATGC CGCGTGTATG AAGAAGGCCT TCGGGTTGTA AAGTACTTTC AGCGGGGAGG AAGGCGRTGC GGTTAATAAC CGCACCGATT GACGTTACCC GCAGAAGAAG CACCGGCTAA CTCCGTGCCA GCAGCCGCGG TAATACGGAG GGTGCAAGCG TTAATCGGAA TTACTGGGCG TAAAGCGCAC GCAGGCGGTC TGTTAAGTCA GATGTGAAAT CCCCGGGCTT AACCTGGGAA CTGCATTTGA AACTGGCAGG CTTGAGTCTT GTAGAGGGGG GTAGAATTCC AGGTGTAGCG GTGAAATGCG TAGAGATCTG GAGGAATACC GGTGGCGAAG GCGGCCCCCT GGACAAAGAC TGACGCTCAG GTGCGAAAGC GTGGGGAGCA AACAGGATTA GATACCCTGG TAGTCCACGC CGTAAACGAT GTCGACTTGG AGGTTGTTCC CTTGAGGAGT GGCTTCCGGA GCTAACGCG

MK070331 – *Bacillus licheniformis* NU 06

AACACGTGGG TAACCTGCCT GTAAGACTGG GATAACTCCG GGAAACCGGG GCTAATACCG GATGCTTCAT TGAACCGCAT GGTTCAATTA TAAAAGGTGG CTTTTAGCTA CCACTTACAG ATGGACCCGC GGCGCATTAG CTAGTTGGTG AGGTAACGGC TCACCAAGGC AACGATGCGT AGCCGACCTG AGAGGGTGAT CGGCCACACT GGGACTGAGA CACGGCCCAG ACTCCTACGG GAGGCAGCAG TAGGGAATCT TCCGCAATGG ACGAAAGTCT GACGGAGCAA CGCCGCGTGA GTGATGAAGG TTTTCGGATC GTAAAACTCT GTTGTTAGGG AAGAACAAGT ACCGTTCGAA TAGGGCGGTA CCTTGACGGT ACCTAACCAG AAAGCCACGG CTAACTACGT GCCAGCAGCC GCGGTAATAC GTAGGTGGCA AGCGTTGTCC GGAATTATTG GGCGTAAAGC GCGCGCAGGC GGTTTCTTAA GTCTGATGTG AAAGCCCCCG GCTCAACCGG GGAGGGTCAT TGGAAACTGG GGAACTTGAG TGCAGAAGAG GAGAGTGGAA TTCCACGTGT AGCGGTGAAA TGCGTAGAGA TGTGGAGGAA CACCAGTGGC GAAGGCGACT CTCTGGTCTG TAACTGACGC TGAGGCGCGA AAGCGTGGGG AGCGAACAGG ATTAGATACC CTGGTAGTCC ACGCCGTAAA CGATGAGTGC TAAGTGTTAG AGGGTTTCCG CCCTTTAGTG CTGCAGCAAA CGCATTAAGC

MK554845 – *Arthrobacter* sp. NU 07

ATTGACCGGC CACCACCCTG GAACTGAGAA CACGGTCCAG ACTCTACGGA GCAGCAGTGG
GGATATGCAC ATTGGCGCAA GCTGATGCAG CGACGCGCGT GAGGGATGAC GCCTTCGGTG
TAACCTCTTT CAGTAGGAAG AAGCCGYAAG GTGACGGTAC TGCAGAAGAA GCGCCGGCTA
ACTACGTGCC AGCAGCCGCG GTAATACGTA GGGCGCAAGC GTTATCCGGA ATTATTGGGC
GTAAAGAGCT CGTAGGCGGT TTGTCGCGTC TGCCGTGAAA GTCCGGGGCT CAACTCCGGA
TCTGCGGTGG GTACGGGCAG ACTAGAGTGA TGTAGGGGAG ACTGGAATTC CTGGTGTAGC
GGTGAAATGC GCAGATATCA GGAGGAACAC CGATGGCGAA GGCAGGTCTC
TGGGCATTAA CTGACGCTGA GGAGCGAAAG CATGGGGAGC GAACAGGATT
AGATACCCTG GTAGTCCATG CCGTAAACGT TGGGCACTAG GTGTGGGGGA CATTCCACGT
TTTCCGCGCC GTAGCTAACG CATTAAGTGC CCCGCCTGGG GAGTACGGCC GCAAGGCTAA
AACTCAAAGG AATTGACGGG GGCCCGCACA AGCGGCGGAG CATGCGGATT AATTCGATGC
AACGCGAAGA ACCTTACCAA GGCTTGACAT GAACCAGACC GGGCTGGAAA CAGTTCTTCC
CCTTTGGGGT TGGTTTACAG GTGGTGCATG GTTGTCGTCA GCTCGTGTCG TGAGATGTTG
GGTTAAGTCC CGCAACGAGC GCAACCCTCG TTCCATGTTG CCAGCGGGTA GTGCCGGGGA
CTCATGGGAG ACTGCCGGGG TCAACTCGGA GGAAGGTGGG GACGACGTCA AATCATCATG
CCCCTTATGT CTTGGGCTTC ACGCATGCTA CAATGGCCGG TACAAAGGGT TGCGATACTG
TGAGGTGGAG CTAATCCCAA AAAGCCGGTC TCAGTTCGGA TTGGGGTCTG CAACTCGACC
CCATGAAGTC GGAGTCGCTA GTAATCGCAG ATCAGCAACG CTGCGGTGAA TACGTTCCCG
GGCCTTGTAC ACACCGCCCG TCAAGTCACG AAAGTTGGTA ACACCCGAAG CCGGTGGCCT
AACCCCTTGT GGGAGGGAGC TGTCGAAG

APPENDIX III

MTS ASSAY RESULTS

The following tables show the MTS data on the crude endophytic and plant samples and their standard deviation.

UMG87 glioblastoma cell line (% Cell viability)

Extract conc.	N1	N2	N3	N4	N5	N6	N7	N16 RFC	N17 RFS	N18 Stem	N19 GF	N20 Leaves	Positive control
100 µg/ml	295.2167	323.516	309.9493	283.606	308.2727	297.4328	298.618	62.05827	184.8716	181.7979	187.2805	189.5159	23.98502
50.0 µg/ml	275.8302	288.5297	285.1669	268.5073	267.1005	286.1305	289.0693	137.894	189.4725	180.6031	181.7497	187.31902	27.29359
25.0 µg/ml	266.1851	286.6604	265.5299	250.9322	251.7319	251.3658	276.7841	170.4522	183.1035	184.3561	187.9501	186.68789	26.12891
12.5 µg/ml	272.9781	280.4648	251.8187	229.6668	227.1616	239.36	269.3937	182.7036	182.8096	183.407	177.4282	181.77382	27.97289
6.25 µg/ml	262.3984	279.9542	257.359	239.4371	235.8527	251.8379	269.2106	187.0685	183.2529	183.4456	182.2797	186.08086	79.65981
3.13 µg/ml	266.1851	275.2521	260.3846	263.5835	247.3478	257.4265	267.3028	183.5275	186.871	185.4112	180.738	179.24933	94.5827

UMG87 glioblastoma cell line (Standard deviation)

Extract conc.	N1	N2	N3	N4	N5	N6	N7	N16 RFC	N17 RFS	N18 Stem	N19 GF	N20 Leaves	Positive control
100 µg/ml	20.85845	31.18465	31.80424	12.78288	7.712971	4.57792	6.254552	7.566241	5.774583	5.136475	6.810814	1.0301703	0.142893
50.0 µg/ml	8.091414	10.63094	6.164733	4.505309	17.65704	29.33154	7.41583	7.317741	2.153586	2.88423	3.420406	2.5987307	0.153965
25.0 µg/ml	13.12686	11.26327	2.805105	6.937518	7.284471	2.666912	4.222435	4.940241	2.772447	5.106496	11.57629	4.8914296	0.147978
12.5 µg/ml	10.93517	15.68222	7.420842	3.399332	6.607188	6.191647	5.61738	3.473734	3.313223	3.217022	6.635723	2.5199951	0.137921
6.25 µg/ml	4.931756	4.055857	16.88647	11.42163	5.496486	2.689428	4.728666	3.204704	7.076773	7.293598	6.365581	2.72514	0.054808
3.13 µg/ml	3.956508	7.484078	9.365784	9.695146	8.921852	5.698091	8.046679	8.429978	3.320119	2.096124	7.367234	9.2345797	0.037693

N1 = *P. ananatis*, N2 = *Xanthomonas* sp., N3 = *P. eucalypti*, N4 = *B. safensis*, N5 = *P. vagans*, N6 = *B. licheniformis*, N7 = *Arthrobacter* sp. N16 = Ripe fruit coat, N17 = Ripe fruit seed, N18 = Stem N19 = Green fruit N20 = Leaves.

A549 Lung carcinoma cells (% Cell viability)

Extract conc.	N1	N2	N3	N4	N5	N6	N7	N16 RFC	N17 RFS	N18 Stem	N19 GF	N20 Leaves	Positive control
100 µg/ml	95.30796	113.3784	86.65743	93.38592	97.22185	99.89421	96.22684	109.0287	104.4642	108.243	102.3627	96.08004	3.556634
50.0 µg/ml	88.94104	122.9342	76.66119	81.40512	84.71636	89.93604	94.96542	104.63	100.9925	111.663	98.71979	135.4234	3.398254
25.0 µg/ml	89.01716	111.2361	83.02268	76.63128	81.74494	80.35575	96.25403	100.5739	100.7968	102.7841	99.43205	115.4092	3.514335
12.5 µg/ml	87.96506	94.47064	77.52298	70.49272	78.38749	92.29577	95.0497	95.13941	93.89973	93.48107	93.87527	135.5947	76.48997
6.25 µg/ml	77.32996	95.50914	71.485	67.12439	77.68338	79.58095	87.92157	92.08372	91.90701	85.60805	89.74574	102.08	88.25541
3.13 µg/ml	89.00085	88.25323	74.04047	75.4188	78.52614	77.28918	92.26858	95.78371	95.83537	102.8439	96.62376	118.8128	97.20227

A549 Lung carcinoma cells (Standard deviation)

Extract conc.	N1	N2	N3	N4	N5	N6	N7	N16 RFC	N17 RFS	N18 Stem	N19 GF	N20 Leaves	Positive control
100 µg/ml	2.039026	6.932403	6.156977	7.02302	2.980133	6.531988	5.393618	6.300817	8.781604	11.82971	14.95554	1.088999	0.019192
50.0 µg/ml	6.879557	5.153689	7.838972	3.360451	6.995908	5.111434	0.497523	7.367424	8.183767	3.863601	3.335218	9.083324	0.022305
25.0 µg/ml	6.657074	3.7973	0.437988	7.825914	4.077358	7.809777	6.729684	7.235712	5.570424	1.532501	10.66331	8.81325	0.13693
12.5 µg/ml	5.111732	6.153112	2.178126	2.357582	1.555534	7.017471	2.109179	3.104787	1.69985	2.854518	8.622794	6.489108	0.158237
6.25 µg/ml	2.973926	7.398051	6.572942	9.725717	3.761654	6.161687	5.919315	1.146079	7.030129	11.15929	4.413464	12.61398	0.051848
3.13 µg/ml	0.566869	5.792864	7.41115	5.142689	2.818782	1.720091	2.72438	2.158061	0.367281	8.382768	11.58166	9.36065	0.111941

N1 = *P. ananatis*, N2 = *Xanthomonas* sp., N3 = *P. eucalypti*, N4 = *B. safensis*, N5 = *P. vagans*, N6 = *B. licheniformis*, N7 = *Arthrobacter* sp. N16 = Ripe fruit coat, N17 = Ripe fruit seed, N18 = Stem N19 = Green fruit N20 = Leaves.

APPENDIX IV

STATISTICAL ANALYSIS DATA

One way ANOVA analysis – Glioblastoma cells

Descriptives								
Cell viability								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C6	13	219.066315	54.5030160	15.1164168	186.130472	252.002158	94.5827	275.2521
C5	13	215.218253	55.2228733	15.3160693	181.847405	248.589101	79.6598	279.9542
C4	13	208.226072	66.3911709	18.4135978	168.106289	248.345855	27.9729	280.4648
C3	13	214.451401	70.2561363	19.4855463	171.996043	256.906760	26.1289	286.6604
C2	13	220.358947	78.8806689	21.8775613	172.691835	268.026058	27.2936	289.0693
C1	13	226.624671	98.3059037	27.2651520	167.219008	286.030334	23.9850	323.5160
Total	78	217.324276	70.0209156	7.9283075	201.536999	233.111554	23.9850	323.5160

N = No of samples/concentration, C1 – C6 = Concentration of samples from 100 – 3.13 µg/mL.

ANOVA					
Cell viability					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2524.695	5	504.939	0.097	0.992
Within Groups	375000.809	72	5208.345		
Total	377525.504	77			

Post Hoc Tests							
Multiple Comparisons							
Dependent Variable:	Cell viability						
(I) Concentration			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	C6	C5	3.8480620	28.3069564	1.000	-79.030862	86.726986
		C4	10.8402429	28.3069564	0.999	-72.038681	93.719166
		C3	4.6149135	28.3069564	1.000	-78.264010	87.493837
		C2	-1.2926316	28.3069564	1.000	-84.171555	81.586292
		C1	-7.5583558	28.3069564	1.000	-90.437279	75.320568
	C5	C6	-3.8480620	28.3069564	1.000	-86.726986	79.030862
		C4	6.9921809	28.3069564	1.000	-75.886743	89.871104
		C3	0.7668515	28.3069564	1.000	-82.112072	83.645775
		C2	-5.1406936	28.3069564	1.000	-88.019617	77.738230
		C1	-11.4064178	28.3069564	0.999	-94.285341	71.472506
	C4	C6	-10.8402429	28.3069564	0.999	-93.719166	72.038681
		C5	-6.9921809	28.3069564	1.000	-89.871104	75.886743
		C3	-6.2253294	28.3069564	1.000	-89.104253	76.653594
		C2	-12.1328745	28.3069564	0.998	-95.011798	70.746049
		C1	-18.3985987	28.3069564	0.987	-101.277522	64.480325
	C3	C6	-4.6149135	28.3069564	1.000	-87.493837	78.264010
		C5	-0.7668515	28.3069564	1.000	-83.645775	82.112072
		C4	6.2253294	28.3069564	1.000	-76.653594	89.104253
		C2	-5.9075451	28.3069564	1.000	-88.786469	76.971378
		C1	-12.1732693	28.3069564	0.998	-95.052193	70.705654

	C2	C6	1.2926316	28.3069564	1.000	-81.586292	84.171555
		C5	5.1406936	28.3069564	1.000	-77.738230	88.019617
		C4	12.1328745	28.3069564	0.998	-70.746049	95.011798
		C3	5.9075451	28.3069564	1.000	-76.971378	88.786469
		C1	-6.2657241	28.3069564	1.000	-89.144648	76.613199
	C1	C6	7.5583558	28.3069564	1.000	-75.320568	90.437279
		C5	11.4064178	28.3069564	0.999	-71.472506	94.285341
		C4	18.3985987	28.3069564	0.987	-64.480325	101.277522
		C3	12.1732693	28.3069564	0.998	-70.705654	95.052193
		C2	6.2657241	28.3069564	1.000	-76.613199	89.144648
Games-Howell	C6	C5	3.8480620	21.5194804	1.000	-62.689632	70.385756
		C4	10.8402429	23.8236572	0.997	-63.051009	84.731495
		C3	4.6149135	24.6615606	1.000	-72.025358	81.255185
		C2	-1.2926316	26.5919865	1.000	-84.363905	81.778642
		C1	-7.5583558	31.1752237	1.000	-106.204285	91.087574
	C5	C6	-3.8480620	21.5194804	1.000	-70.385756	62.689632
		C4	6.9921809	23.9508364	1.000	-67.264395	81.248757
		C3	0.7668515	24.7844406	1.000	-76.217364	77.751067
		C2	-5.1406936	26.7059856	1.000	-88.512977	78.231590
		C1	-11.4064178	31.2725198	0.999	-110.278741	87.465905
	C4	C6	-10.8402429	23.8236572	0.997	-84.731495	63.051009
		C5	-6.9921809	23.9508364	1.000	-81.248757	67.264395
		C3	-6.2253294	26.8094591	1.000	-89.140103	76.689444
		C2	-12.1328745	28.5952491	0.998	-100.759433	76.493684
		C1	-18.3985987	32.9005942	0.993	-121.299541	84.502343
	C3	C6	-4.6149135	24.6615606	1.000	-81.255185	72.025358

		C5	-0.7668515	24.7844406	1.000	-77.751067	76.217364
		C4	6.2253294	26.8094591	1.000	-76.689444	89.140103
		C2	-5.9075451	29.2969999	1.000	-96.590852	84.775762
		C1	-12.1732693	33.5123116	0.999	-116.688803	92.342264
	C2	C6	1.2926316	26.5919865	1.000	-81.778642	84.363905
		C5	5.1406936	26.7059856	1.000	-78.231590	88.512977
		C4	12.1328745	28.5952491	0.998	-76.493684	100.759433
		C3	5.9075451	29.2969999	1.000	-84.775762	96.590852
		C1	-6.2657241	34.9573483	1.000	-114.769417	102.237969
	C1	C6	7.5583558	31.1752237	1.000	-91.087574	106.204285
		C5	11.4064178	31.2725198	0.999	-87.465905	110.278741
		C4	18.3985987	32.9005942	0.993	-84.502343	121.299541
		C3	12.1732693	33.5123116	0.999	-92.342264	116.688803
		C2	6.2657241	34.9573483	1.000	-102.237969	114.769417

Homogeneous Subsets

Cell viability

Concentration		N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C4	13	208.226072
	C3	13	214.451401
	C5	13	215.218253
	C6	13	219.066315
	C2	13	220.358947
	C1	13	226.624671

	Sig.		0.987
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 13.000.			

One way ANOVA analysis – Lung carcinoma cells

Descriptives								
Cell viability								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C6	13	90.915314	12.6399893	3.5057023	83.277045	98.553583	74.0405	118.8128
C5	13	85.101098	9.9409334	2.7571189	79.093852	91.108344	67.1244	102.0800
C4	13	91.128037	15.9153164	4.4141146	81.510508	100.745567	70.4927	135.5947
C3	13	87.751713	28.0179607	7.7707842	70.820629	104.682797	3.5143	115.4092
C2	13	91.875869	31.3418641	8.6926691	72.936170	110.815568	3.3983	135.4234
C1	13	92.754444	27.7697699	7.7019484	75.973340	109.535548	3.5566	113.3784
Total	78	89.921079	21.9661593	2.4871778	84.968476	94.873683	3.3983	135.5947

N = No of samples/concentration, C1 – C6 = Concentration of samples from 100 – 3.13 µg/mL.

ANOVA					
Cell viability					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	549.026	5	109.805	0.216	0.955
Within Groups	36604.410	72	508.395		
Total	37153.436	77			

Post Hoc Tests							
Multiple Comparisons							
Dependent Variable:	Cell viability						
(I) Concentration			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	C6	C5	5.8142162	8.8438991	0.986	-20.079519	31.707951
		C4	-0.2127228	8.8438991	1.000	-26.106458	25.681012
		C3	3.1636015	8.8438991	0.999	-22.730134	29.057337
		C2	-0.9605545	8.8438991	1.000	-26.854290	24.933181
		C1	-1.8391295	8.8438991	1.000	-27.732865	24.054606
	C5	C6	-5.8142162	8.8438991	0.986	-31.707951	20.079519
		C4	-6.0269390	8.8438991	0.983	-31.920674	19.866796
		C3	-2.6506147	8.8438991	1.000	-28.544350	23.243121
		C2	-6.7747707	8.8438991	0.972	-32.668506	19.118965
		C1	-7.6533458	8.8438991	0.953	-33.547081	18.240390
	C4	C6	0.2127228	8.8438991	1.000	-25.681012	26.106458
		C5	6.0269390	8.8438991	0.983	-19.866796	31.920674
		C3	3.3763243	8.8438991	0.999	-22.517411	29.270060
		C2	-0.7478317	8.8438991	1.000	-26.641567	25.145904
		C1	-1.6264067	8.8438991	1.000	-27.520142	24.267329
	C3	C6	-3.1636015	8.8438991	0.999	-29.057337	22.730134
		C5	2.6506147	8.8438991	1.000	-23.243121	28.544350
		C4	-3.3763243	8.8438991	0.999	-29.270060	22.517411
		C2	-4.1241560	8.8438991	0.997	-30.017891	21.769579
		C1	-5.0027311	8.8438991	0.993	-30.896466	20.891004
	C2	C6	0.9605545	8.8438991	1.000	-24.933181	26.854290

		C5	6.7747707	8.8438991	0.972	-19.118965	32.668506
		C4	0.7478317	8.8438991	1.000	-25.145904	26.641567
		C3	4.1241560	8.8438991	0.997	-21.769579	30.017891
		C1	-0.8785751	8.8438991	1.000	-26.772310	25.015160
	C1	C6	1.8391295	8.8438991	1.000	-24.054606	27.732865
		C5	7.6533458	8.8438991	0.953	-18.240390	33.547081
		C4	1.6264067	8.8438991	1.000	-24.267329	27.520142
		C3	5.0027311	8.8438991	0.993	-20.891004	30.896466
		C2	0.8785751	8.8438991	1.000	-25.015160	26.772310
Games-Howell	C6	C5	5.8142162	4.4600059	0.780	-8.038948	19.667381
		C4	-0.2127228	5.6368746	1.000	-17.715178	17.289732
		C3	3.1636015	8.5249654	0.999	-24.164402	30.491605
		C2	-0.9605545	9.3729635	1.000	-31.208490	29.287381
		C1	-1.8391295	8.4622667	1.000	-28.951261	25.273002
	C5	C6	-5.8142162	4.4600059	0.780	-19.667381	8.038948
		C4	-6.0269390	5.2044319	0.851	-22.375639	10.321761
		C3	-2.6506147	8.2454103	0.999	-29.445827	24.144597
		C2	-6.7747707	9.1194408	0.973	-36.570721	23.021179
		C1	-7.6533458	8.1805693	0.931	-34.225688	18.918996
	C4	C6	0.2127228	5.6368746	1.000	-17.289732	17.715178
		C5	6.0269390	5.2044319	0.851	-10.321761	22.375639
		C3	3.3763243	8.9369734	0.999	-24.859462	31.612111
		C2	-0.7478317	9.7492001	1.000	-31.768967	30.273303
		C1	-1.6264067	8.8771852	1.000	-29.657770	26.404957
	C3	C6	-3.1636015	8.5249654	0.999	-30.491605	24.164402
		C5	2.6506147	8.2454103	0.999	-24.144597	29.445827

		C4	-3.3763243	8.9369734	0.999	-31.612111	24.859462
		C2	-4.1241560	11.6596562	0.999	-40.211965	31.963653
		C1	-5.0027311	10.9409824	0.997	-38.831716	28.826254
	C2	C6	0.9605545	9.3729635	1.000	-29.287381	31.208490
		C5	6.7747707	9.1194408	0.973	-23.021179	36.570721
		C4	0.7478317	9.7492001	1.000	-30.273303	31.768967
		C3	4.1241560	11.6596562	0.999	-31.963653	40.211965
		C1	-0.8785751	11.6138928	1.000	-36.830762	35.073612
	C1	C6	1.8391295	8.4622667	1.000	-25.273002	28.951261
		C5	7.6533458	8.1805693	0.931	-18.918996	34.225688
		C4	1.6264067	8.8771852	1.000	-26.404957	29.657770
		C3	5.0027311	10.9409824	0.997	-28.826254	38.831716
		C2	0.8785751	11.6138928	1.000	-35.073612	36.830762

Homogeneous Subsets			
Cell viability			
Concentration		N	Subset for alpha = 0.05
			1
Tukey HSD[a]	C5	13	85.101098
	C3	13	87.751713
	C6	13	90.915314
	C4	13	91.128037
	C2	13	91.875869
	C1	13	92.754444
	Sig.		0.953

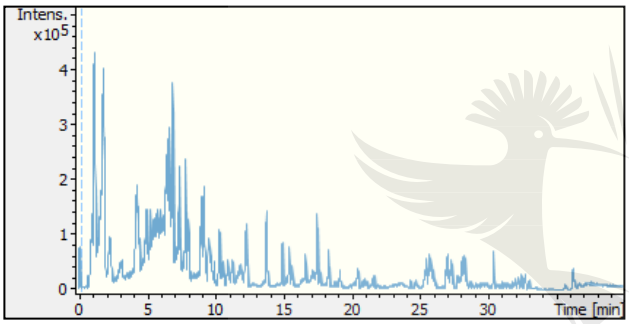
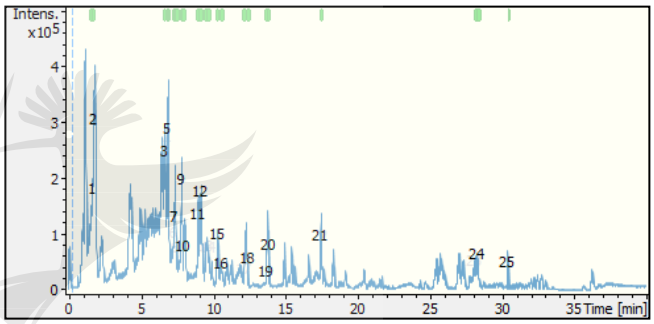
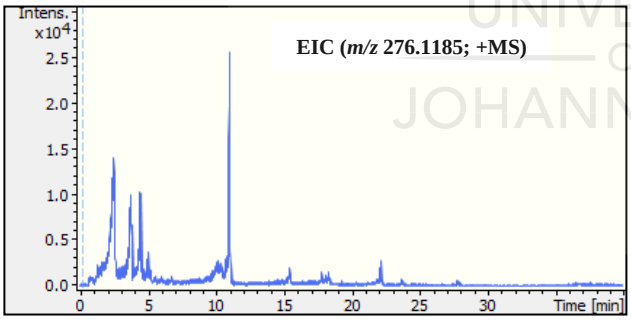
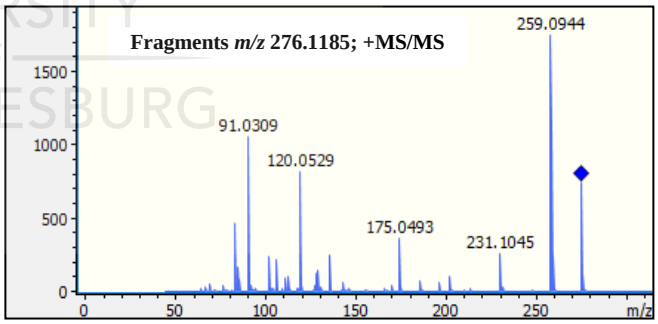
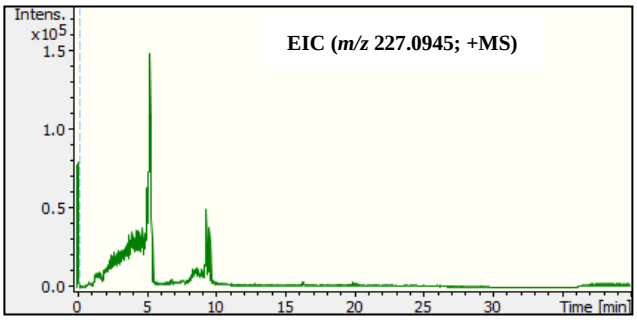
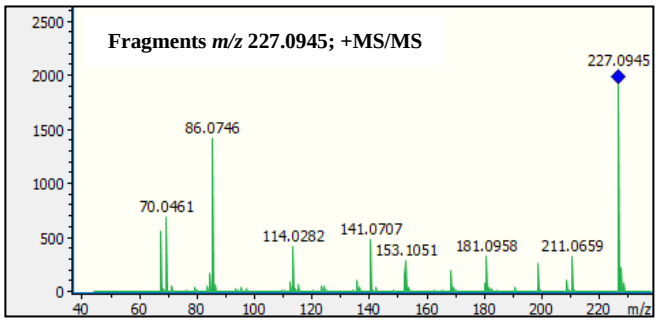
Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 13.000.

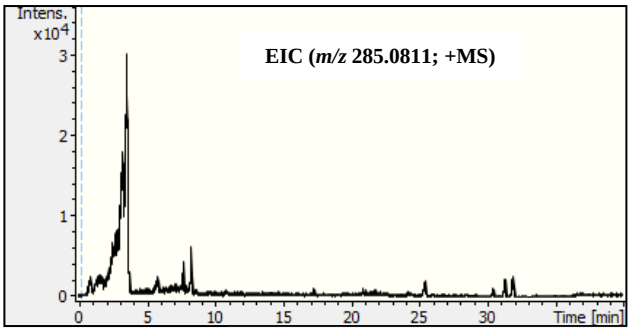
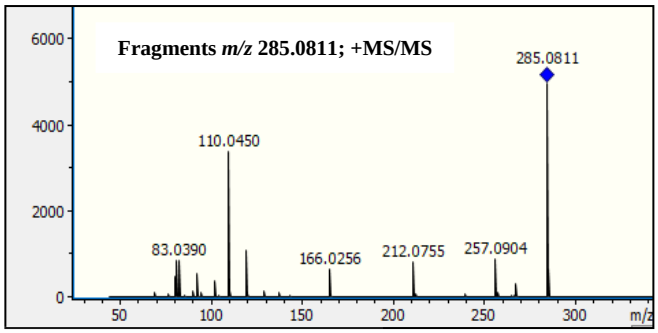
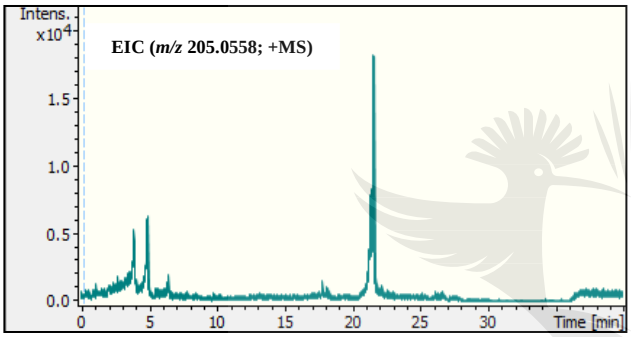
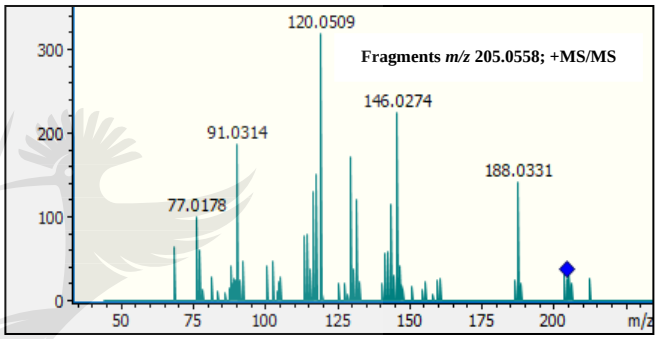
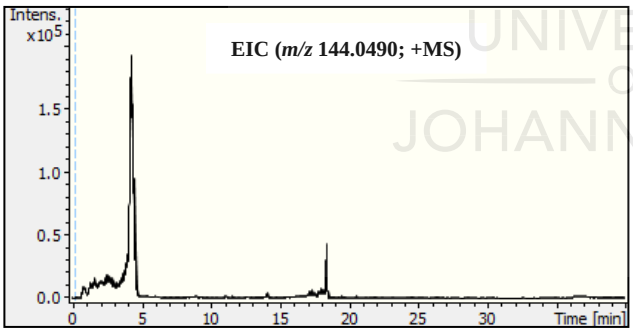
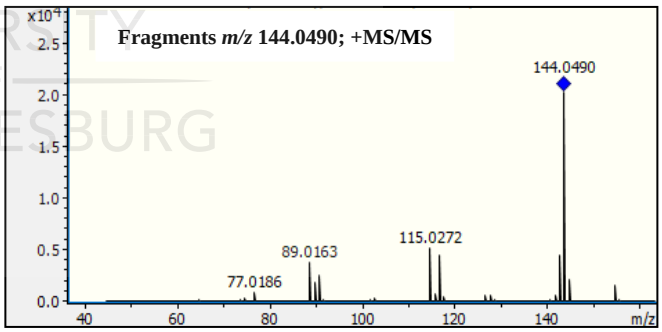
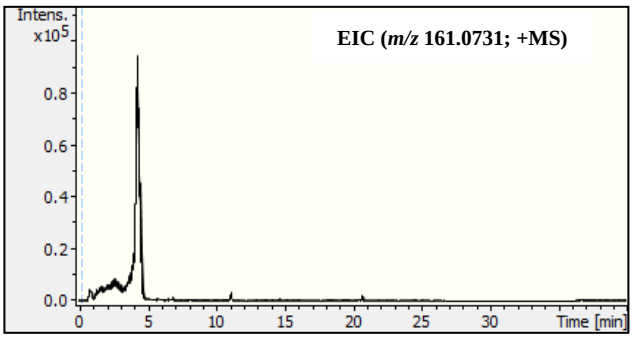
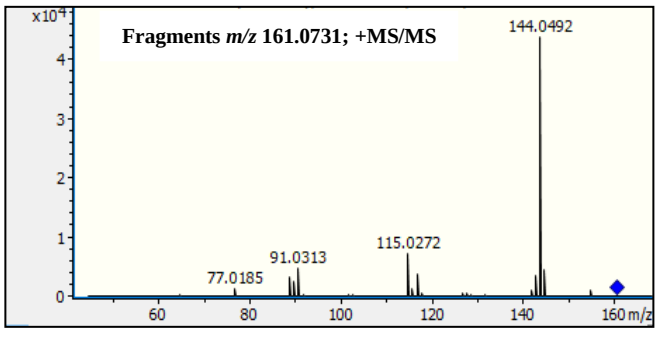
APPENDIX V

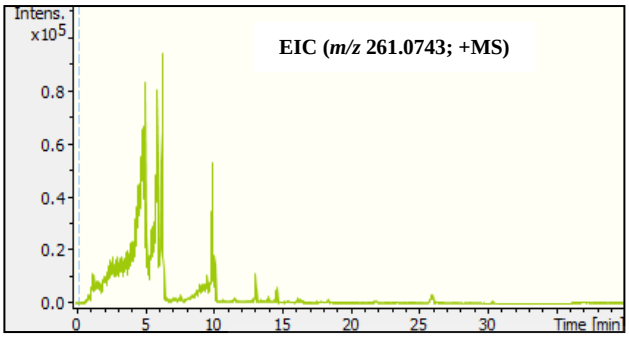
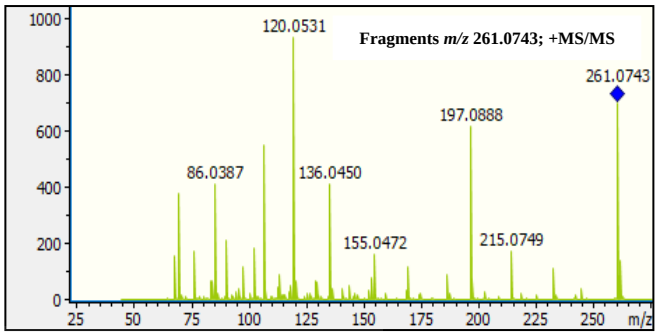
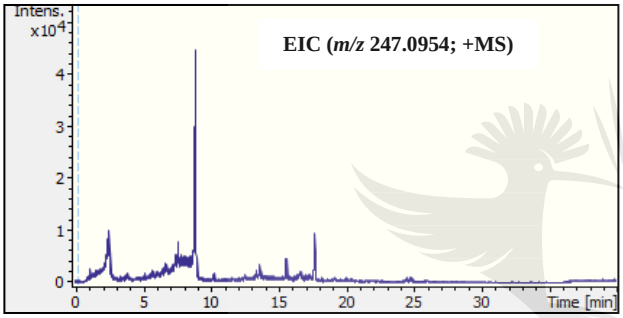
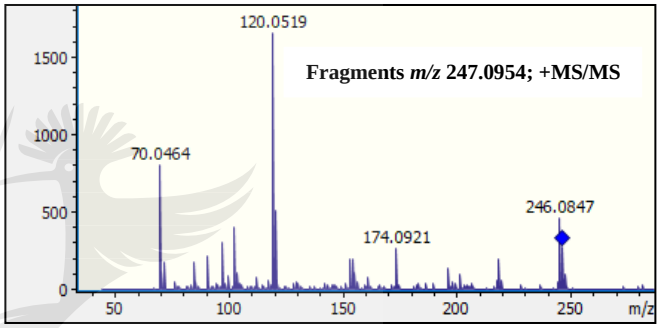
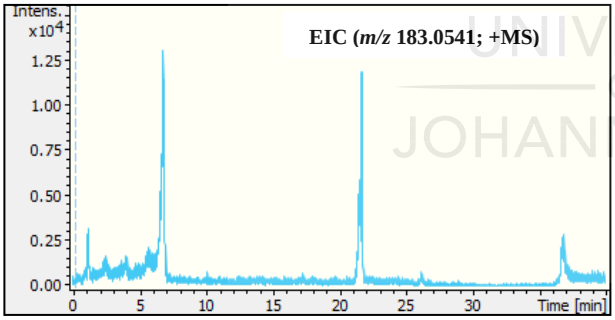
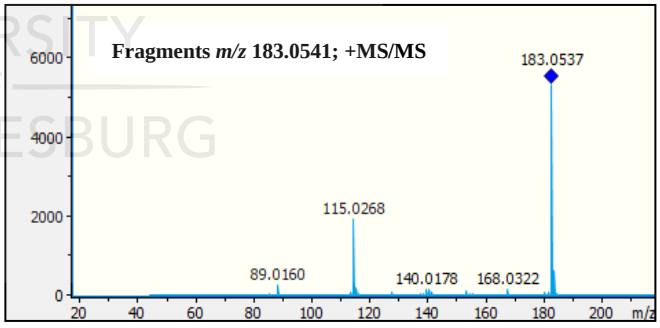
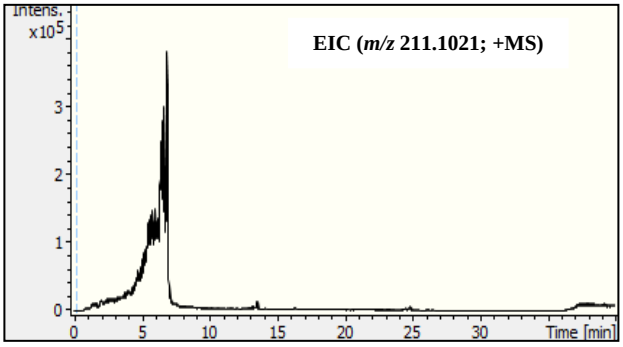
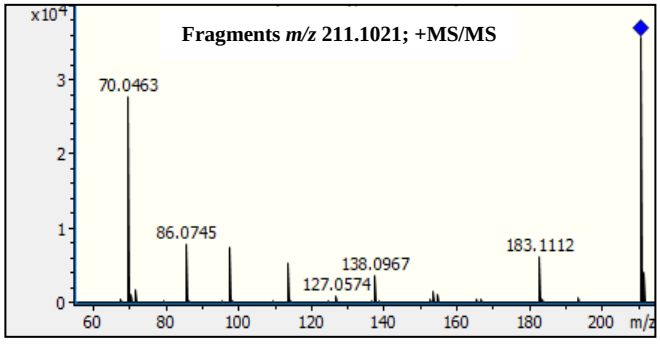
UNTARGETED SECONDARY METABOLITE PROFILING OF PLANTS AND ENDOPHYTES

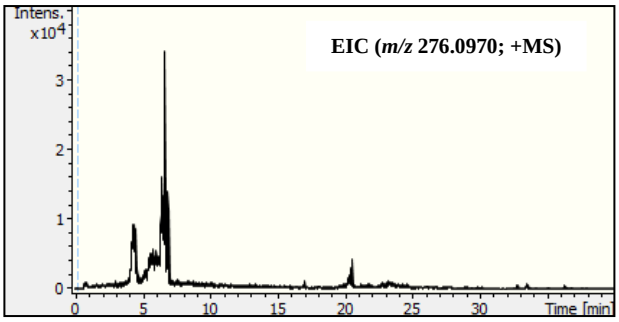
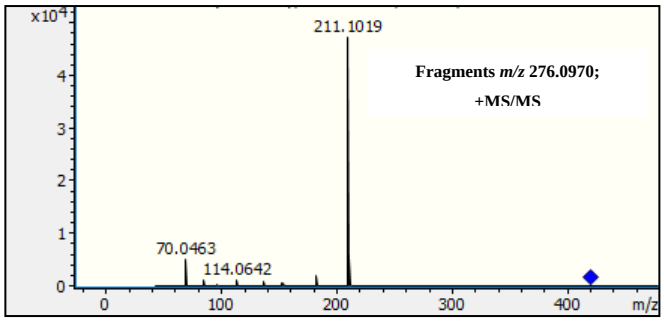
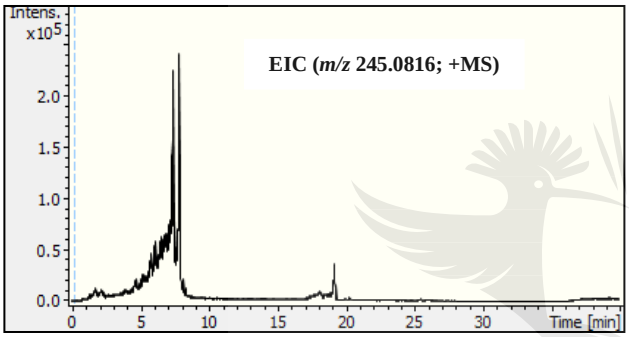
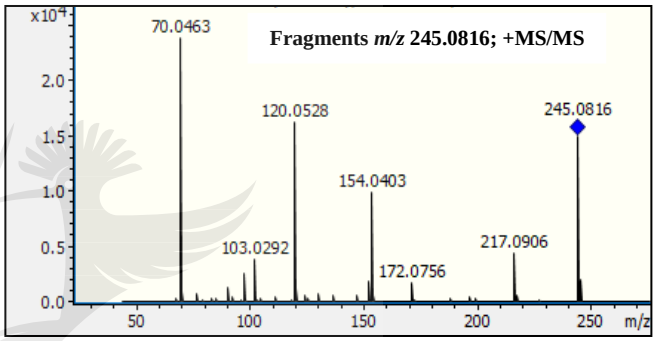
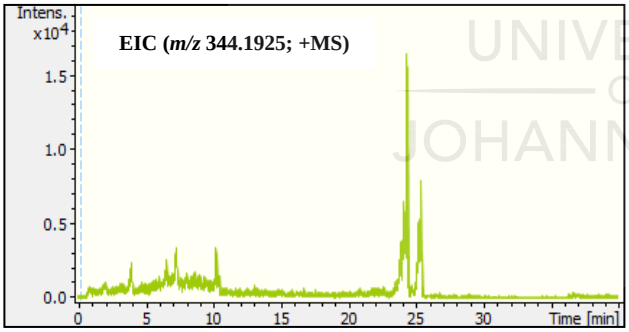
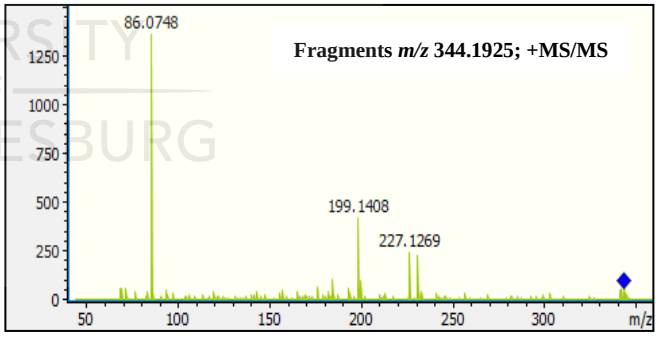
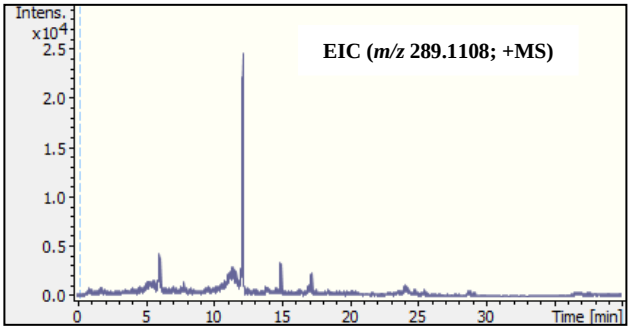
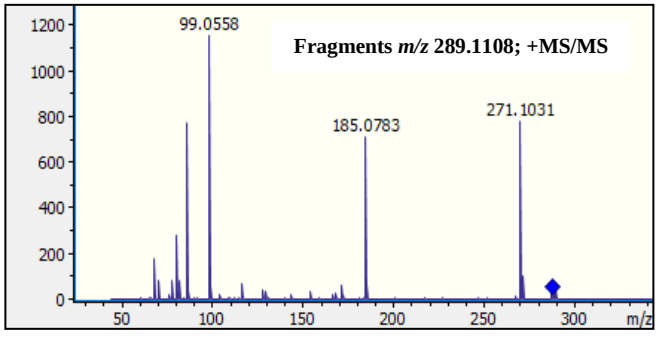
The figures shown in tables A1 – A13 below were obtained from the Bruker data analysis software used in this study. They show the +MS and +MS/MS chromatogram of the compounds identified in this study and reported in tables 5.1 and 5.2 in the Chapter 5 section of this thesis.

Table A1: Extracted ion chromatogram (EIC) of secondary metabolites from *Pantoea ananatis* – NU 01 (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Dubinidine	
	
Genipin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Xanthosine	
	
Droserone	
	
4-Methyl-5-(2'-hydroxyethyl)-thiazole	
	
Anatabine	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Khellin	
	
(R)-Columbianetin	
	
1-Methyluric acid	
	
Sinapyl alcohol	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Eupaformosanin	
	
Fulvoplumierin	
	
(-)-Laudanidine	
	
Asebogenin	
	

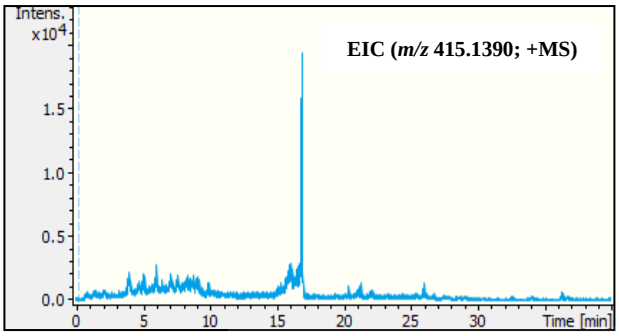
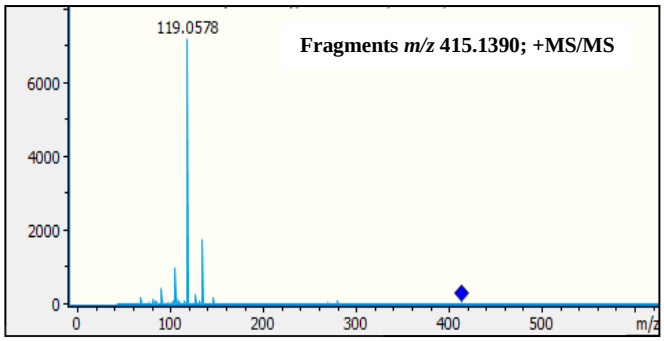
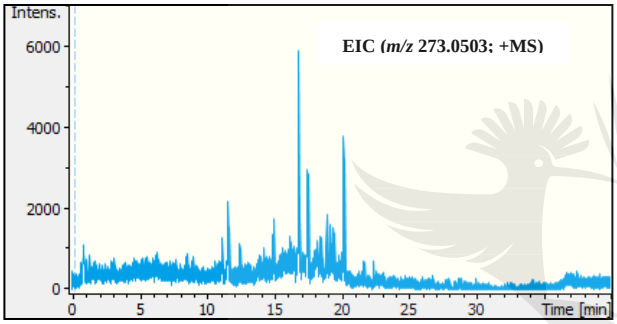
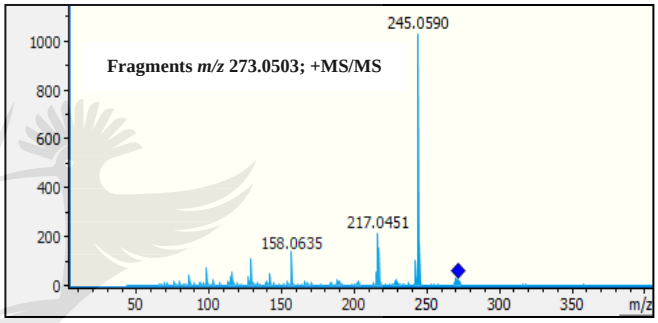
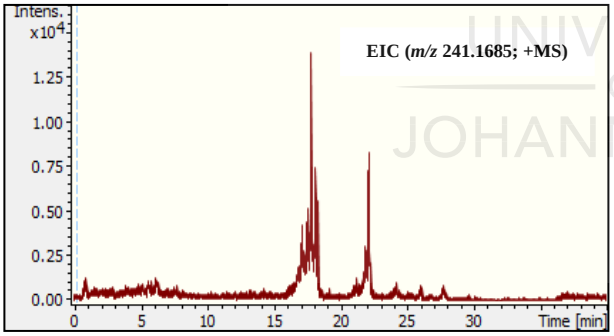
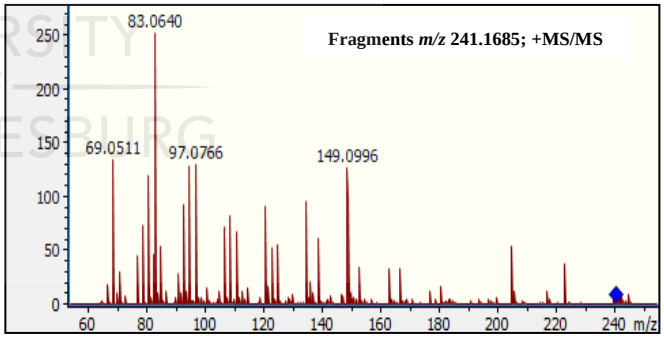
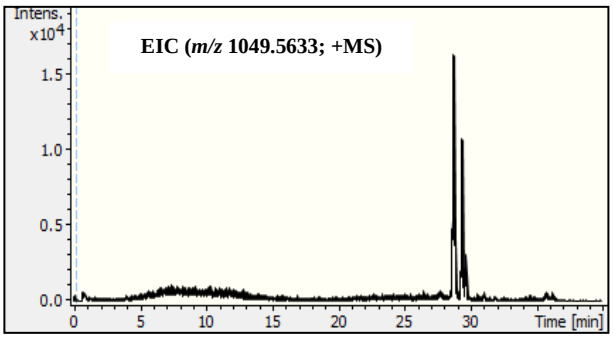
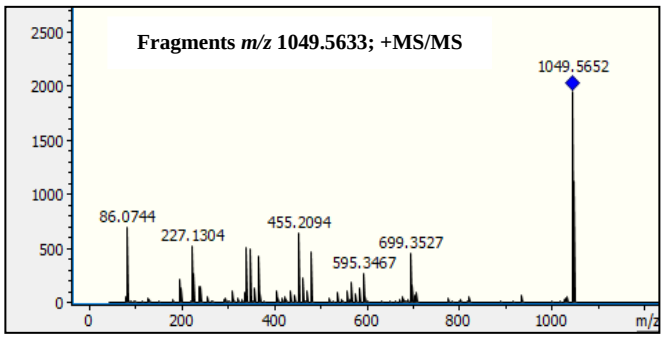
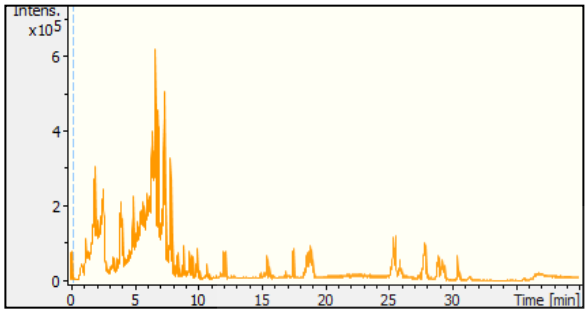
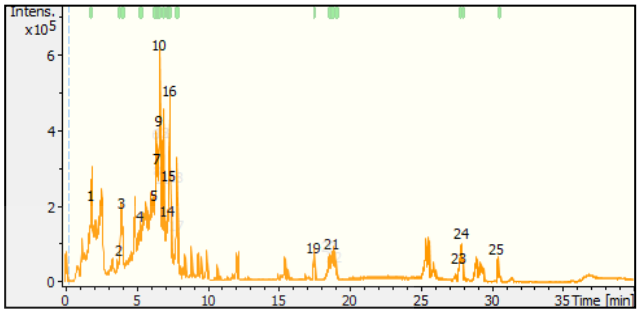
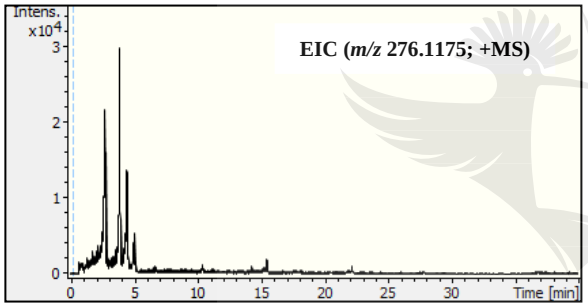
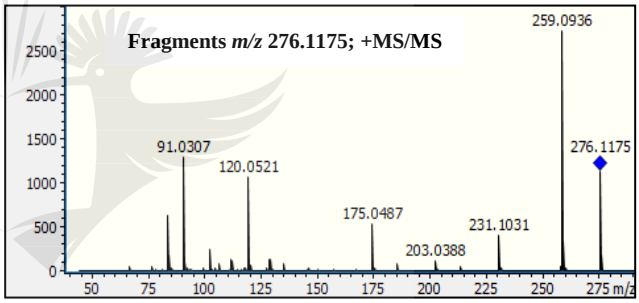
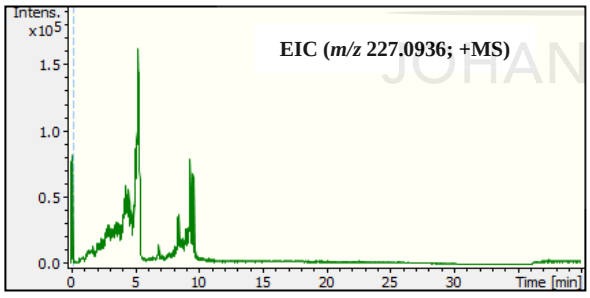
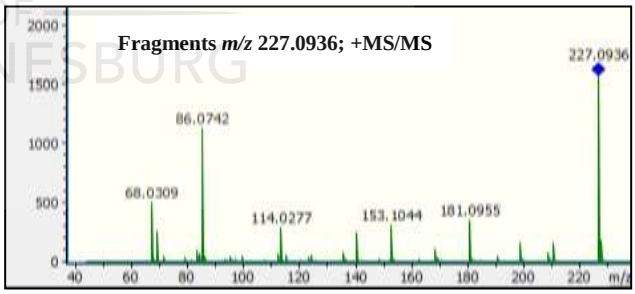
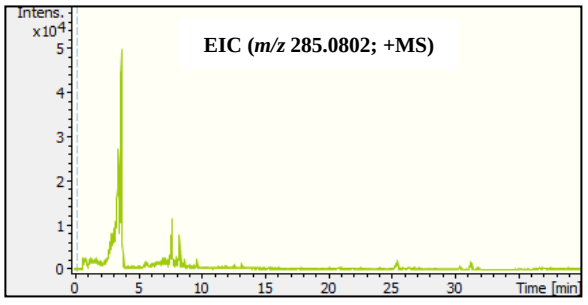
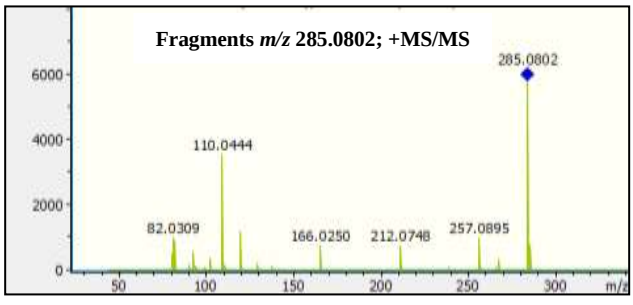
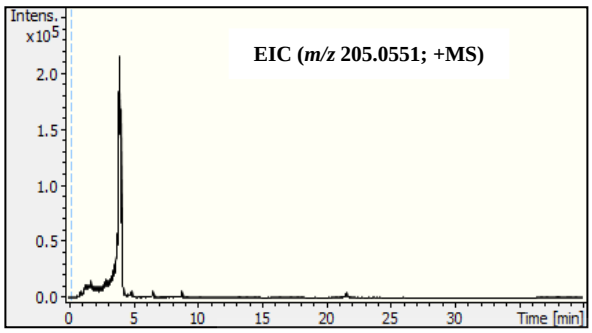
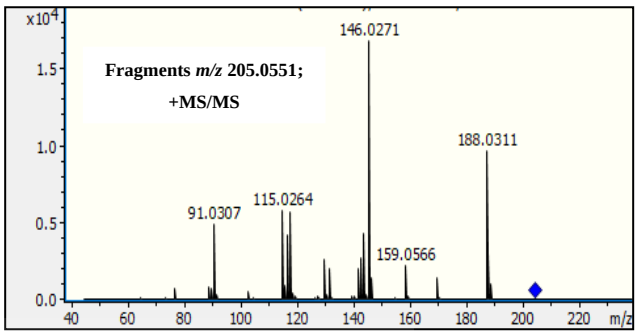
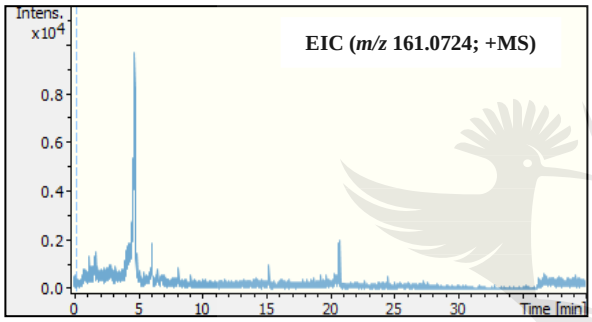
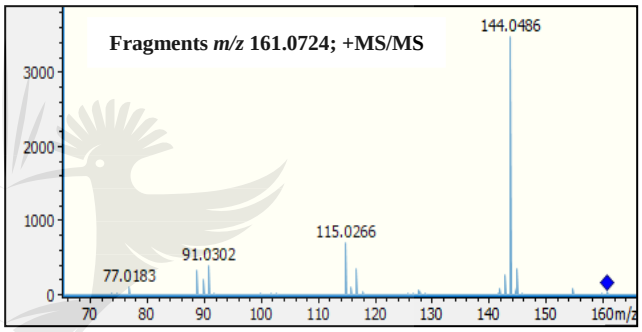
EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Picropodophyllin	
	
Naringenin	
	
Borrerine	
	
Protodioscin	
	

Table A2: Extracted ion chromatogram (EIC) of secondary metabolites from *Xanthomonas sp.* – NU 02 (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Dubinidine	
	
Genipin	
	
Xanthosine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Droserone	
	
Anatabine	
	
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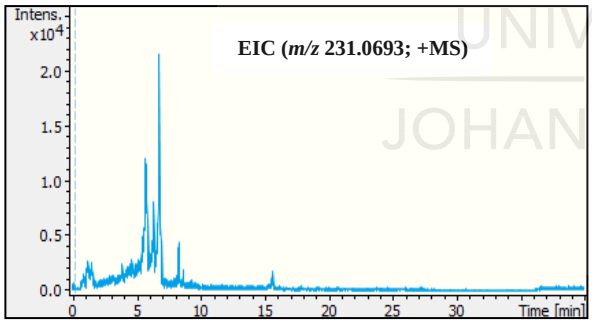
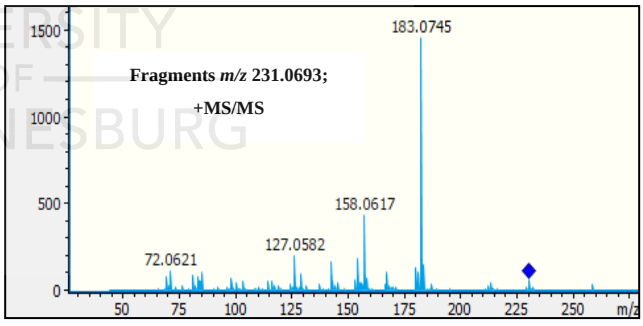
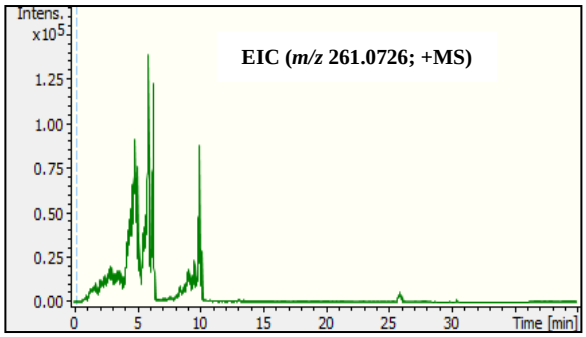
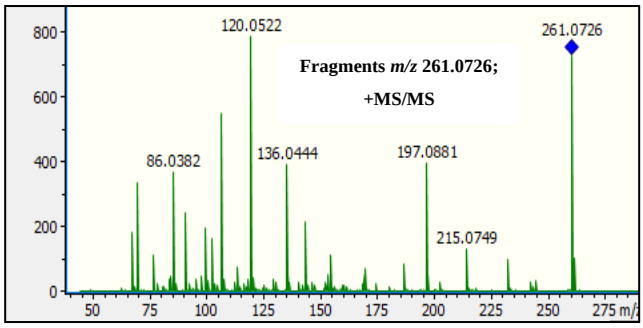
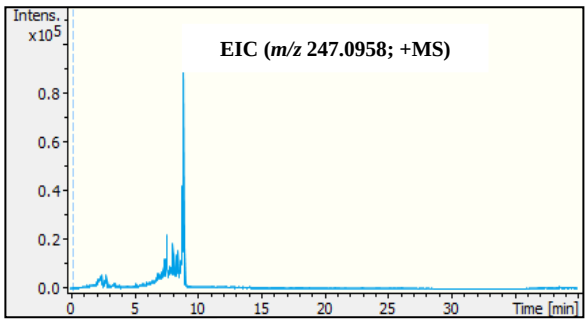
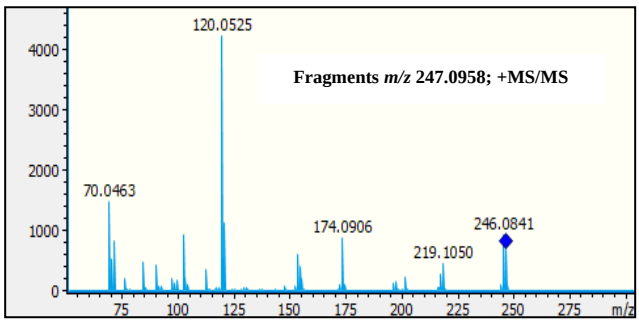
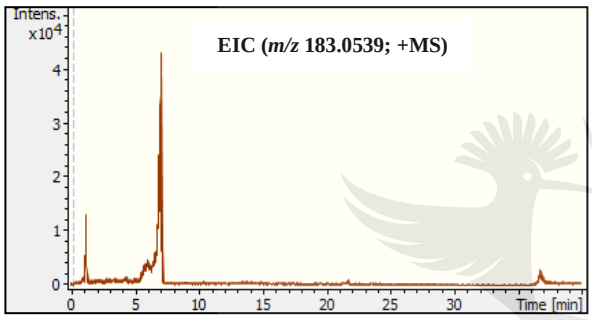
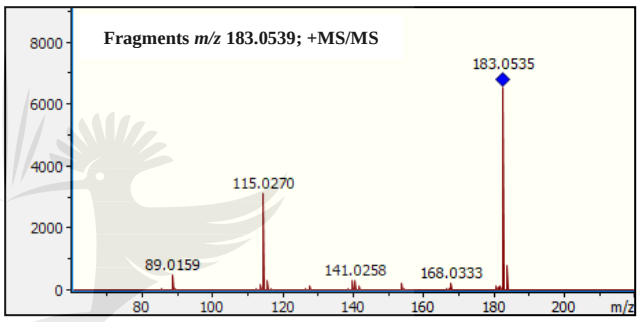
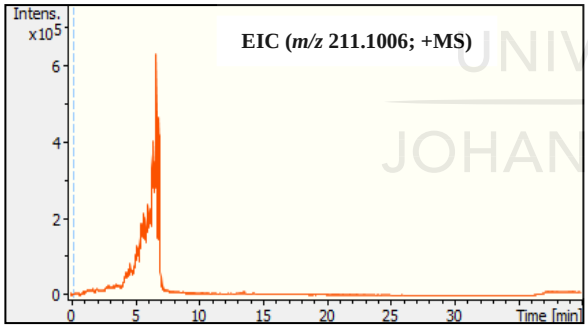
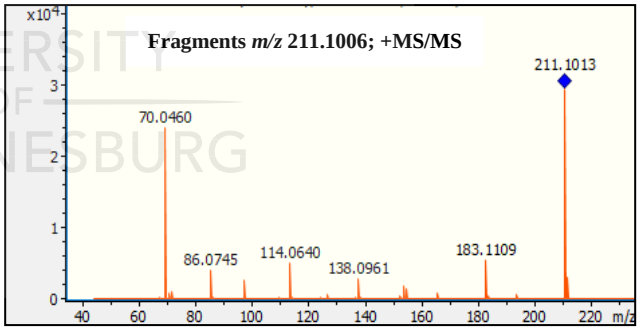
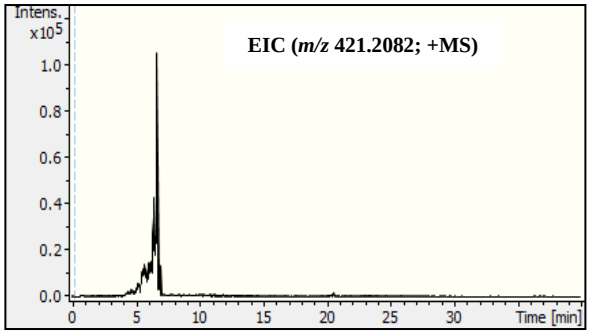
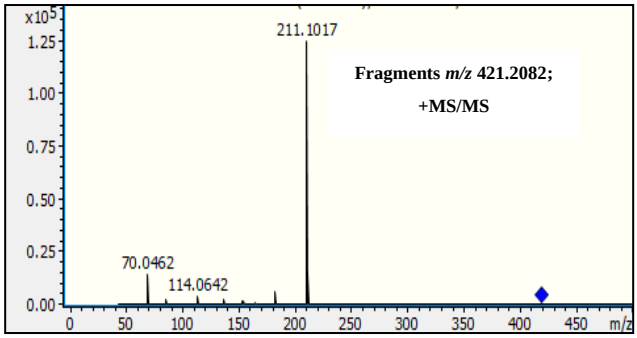
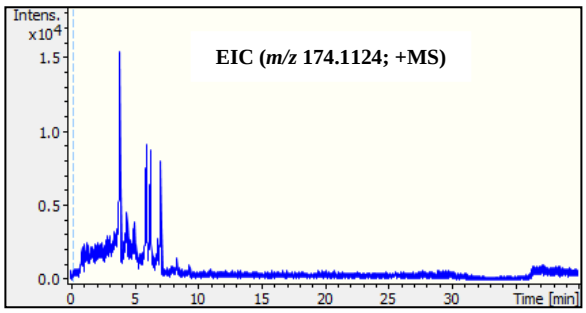
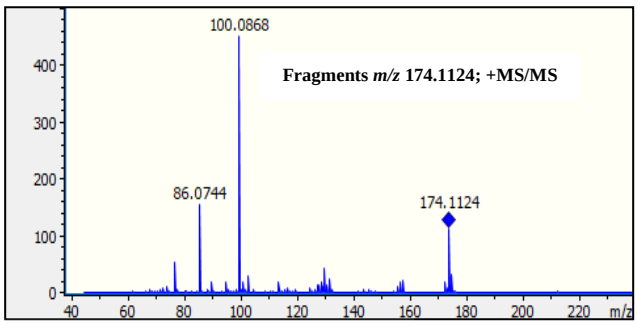
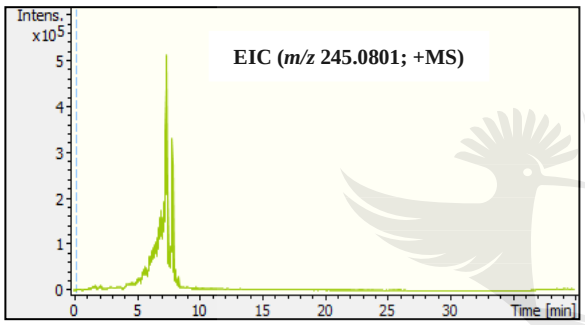
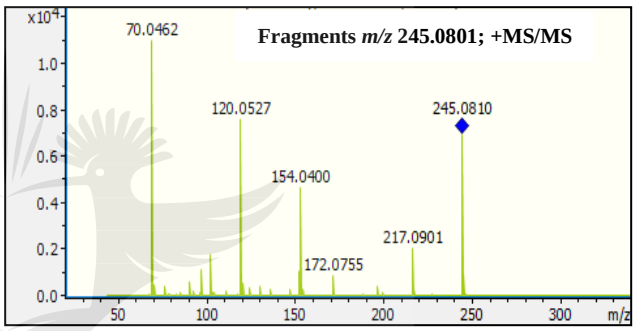
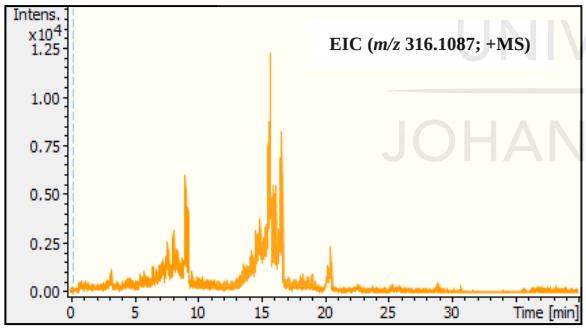
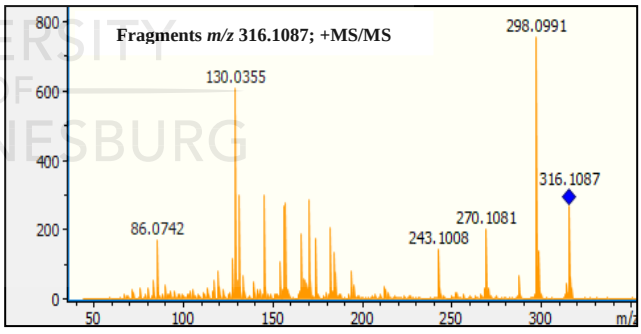
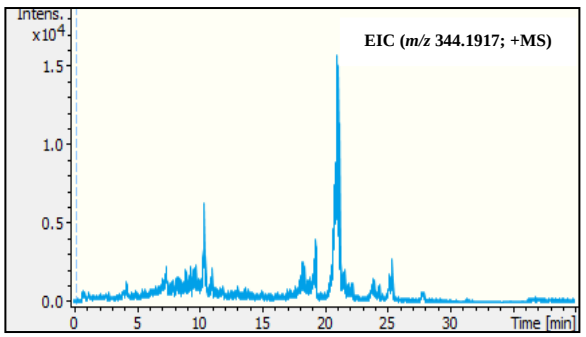
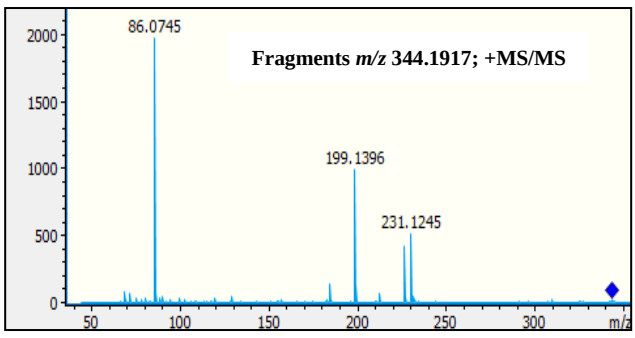
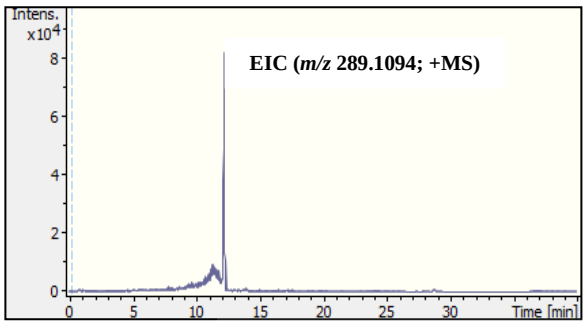
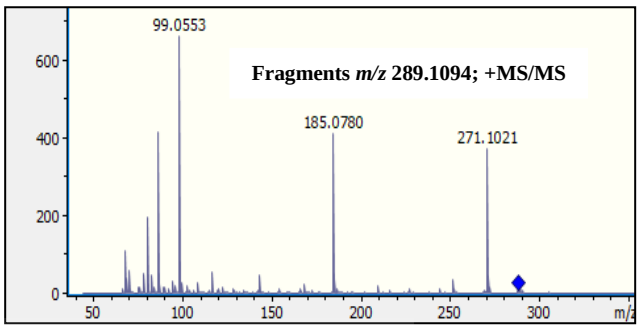
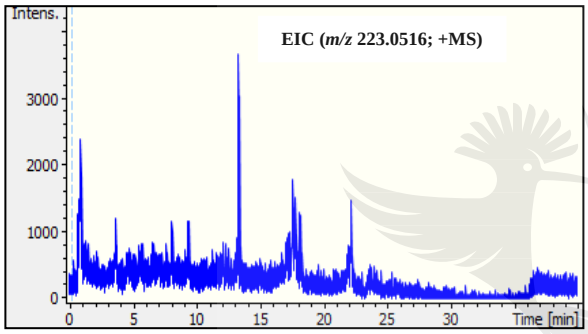
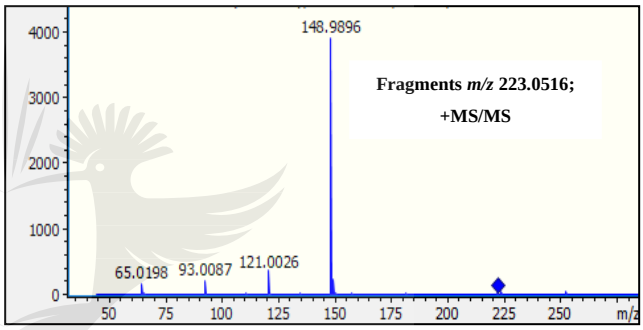
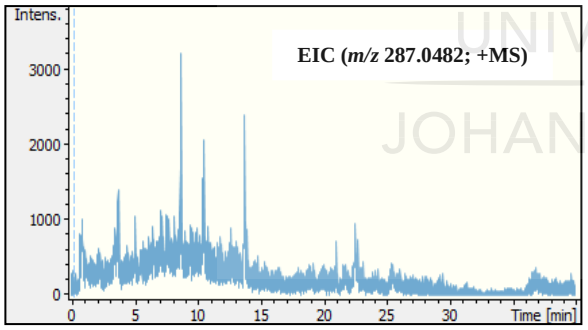
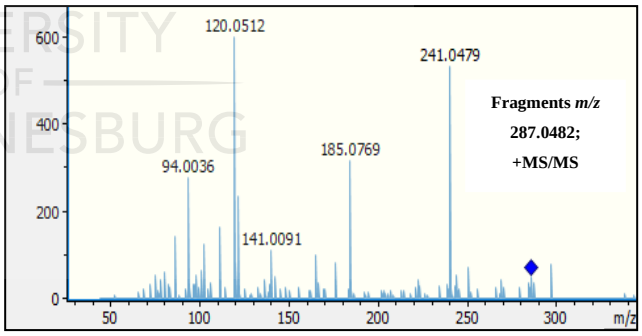
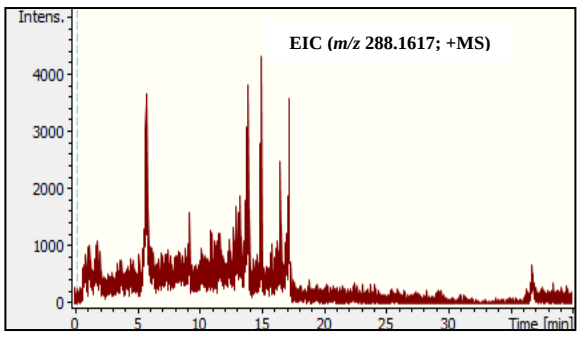
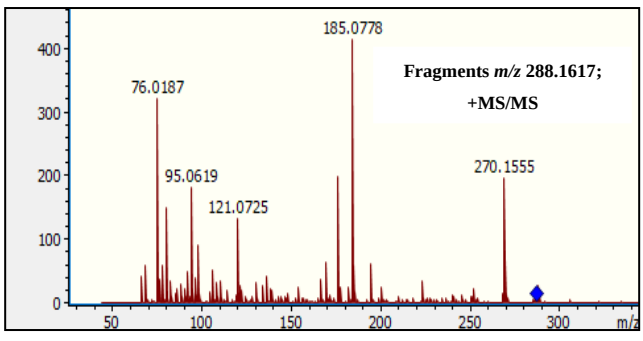
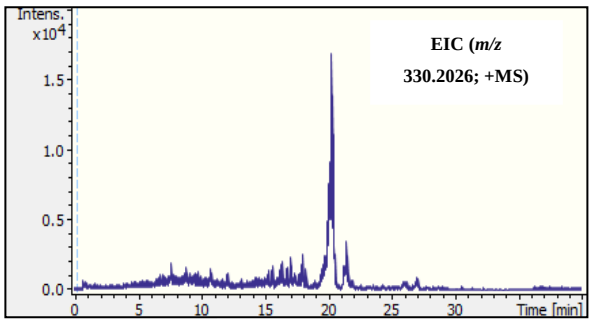
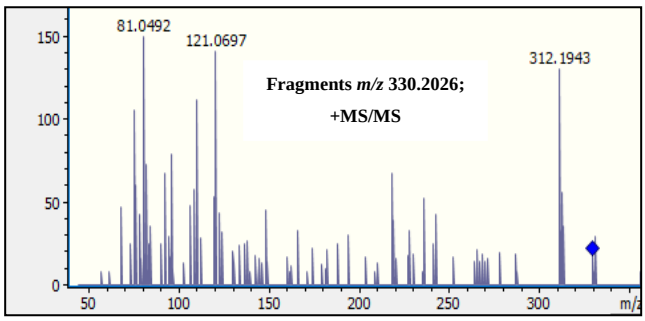
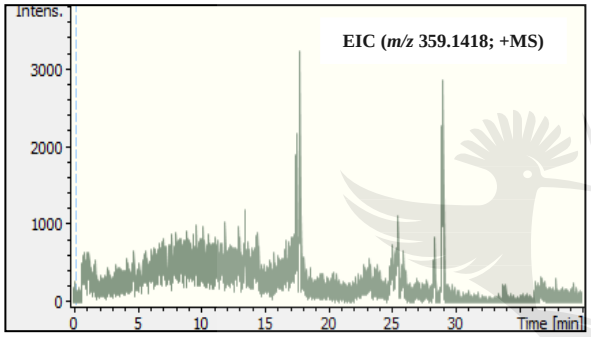
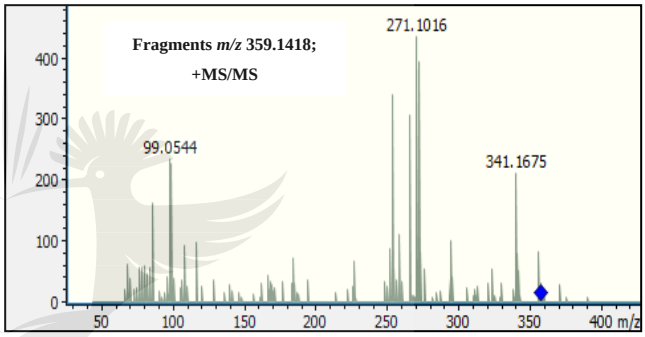
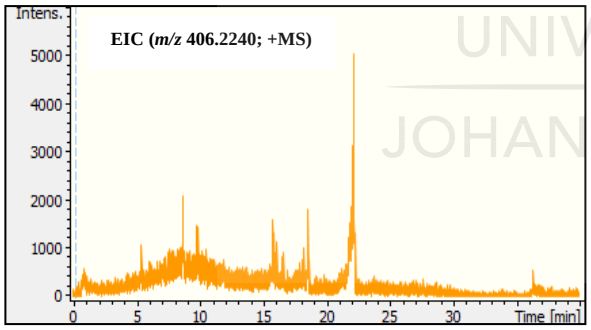
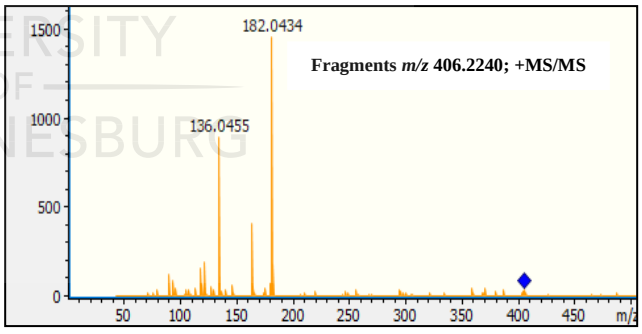
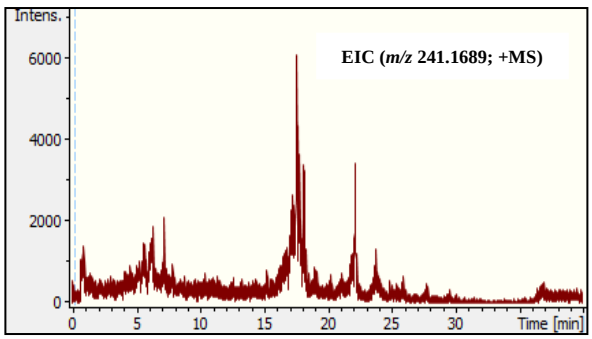
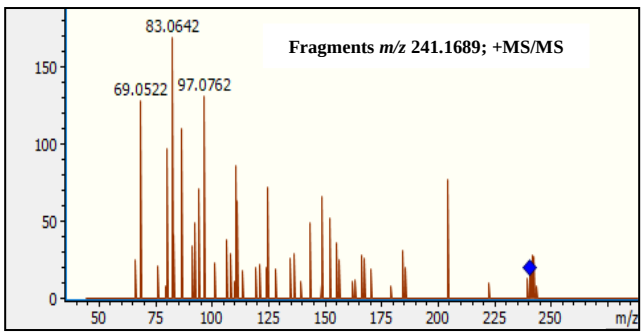
	
Khellin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
(R)-Columbianetin	
	
1-Methyluric acid	
	
Sinapyl alcohol	
	
Eupaformosanin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Swainsonine	
	
Fulvoplumierin	
	
Rosinidin	
	
(-)-Laudanidine	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Asebogenin	
	
Leptodactylone	
	
Kaempferol	
	
Mesembrenone	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Hetisine	
	
Columbin	
	
Icacine	
	
Borrerine	
	

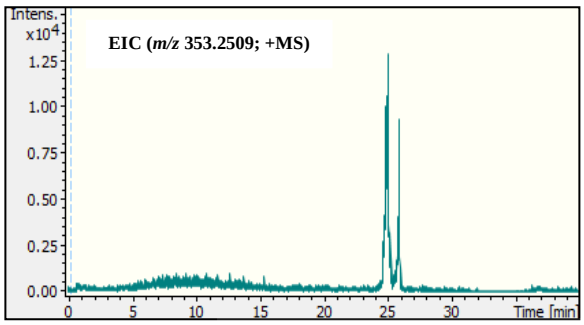
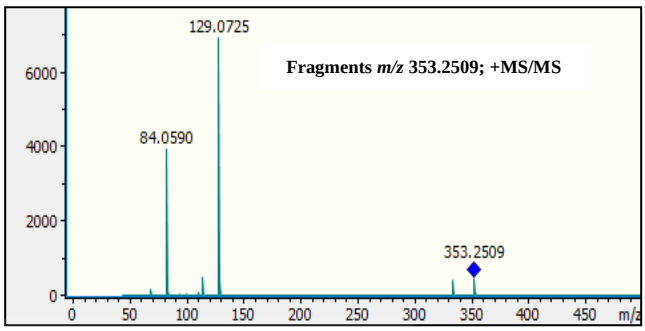
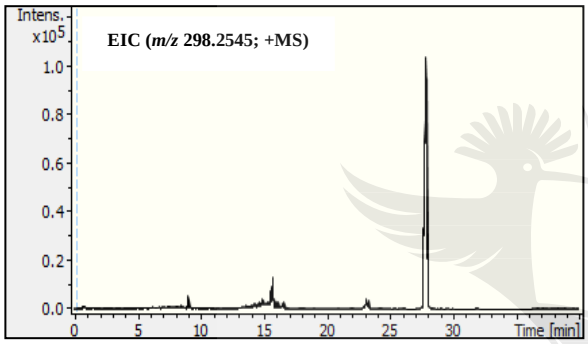
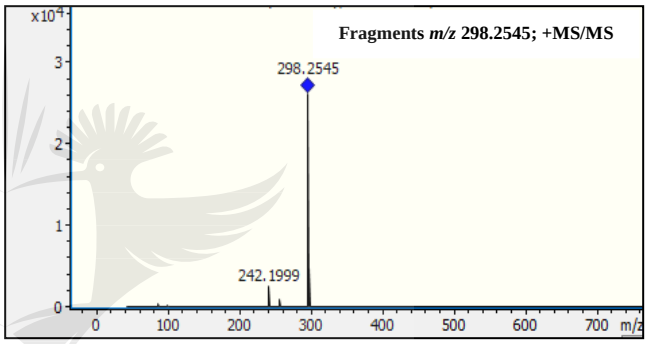
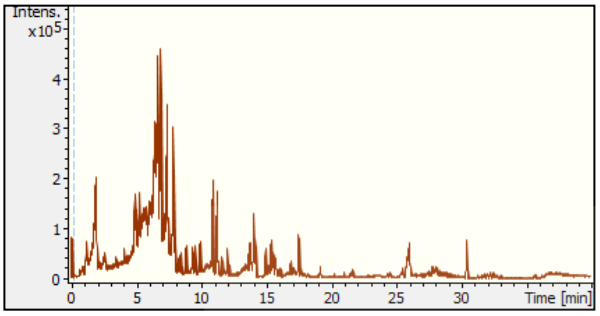
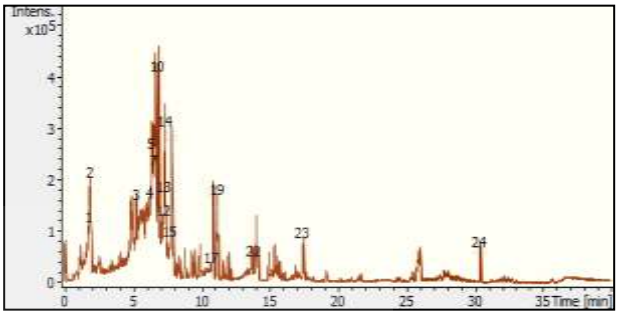
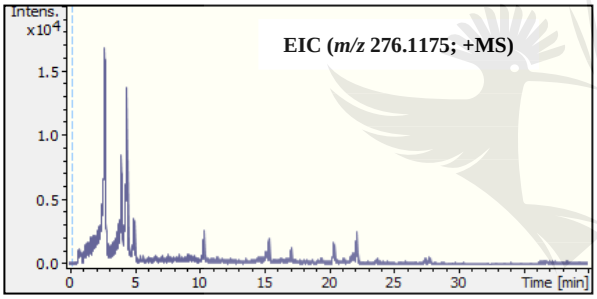
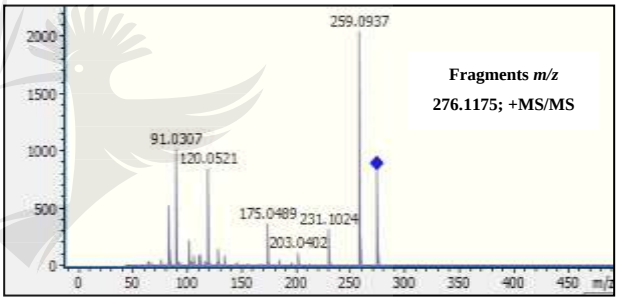
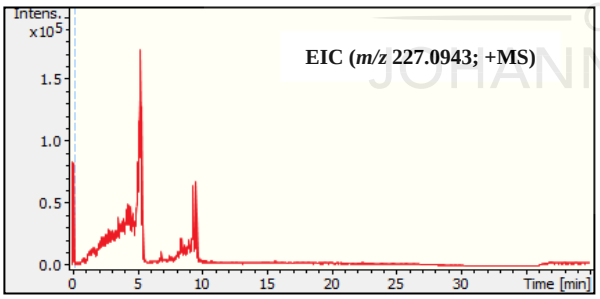
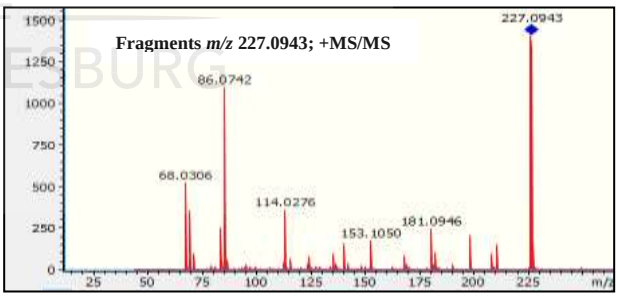
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Montanol 	
Cassine 	

Table A3: Extracted ion chromatogram (EIC) of secondary metabolites from *Pantoea eucalypti*. – NU 03 (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Dubinidine	
	
Genipin	
	
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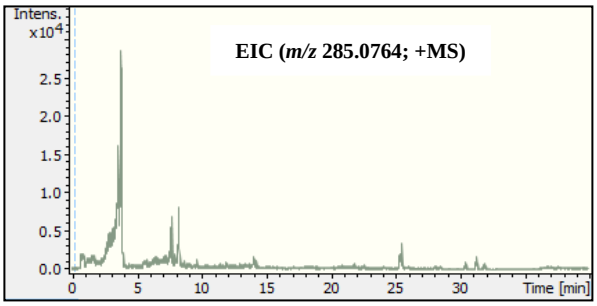
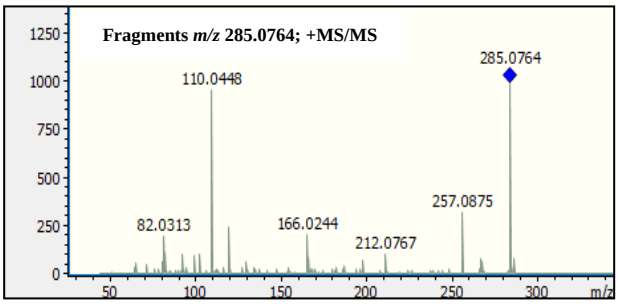
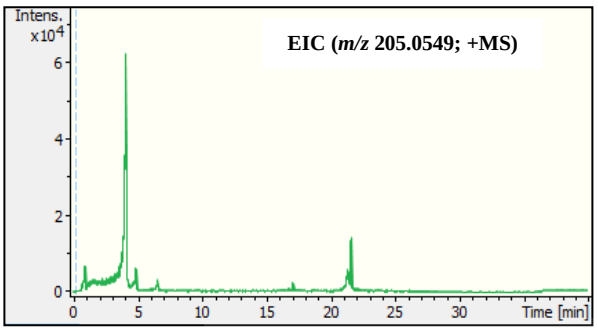
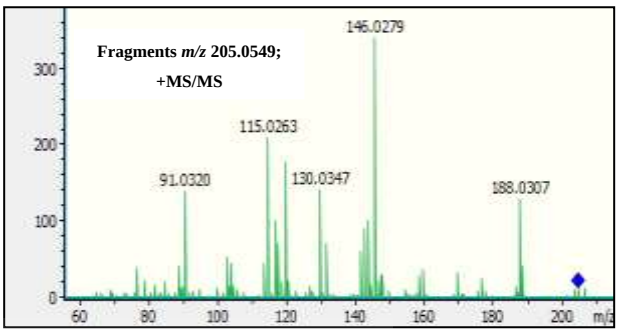
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Droserone	
	
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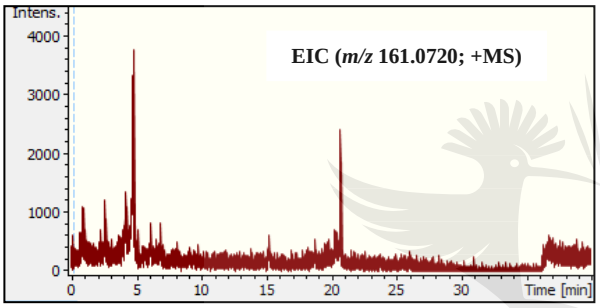	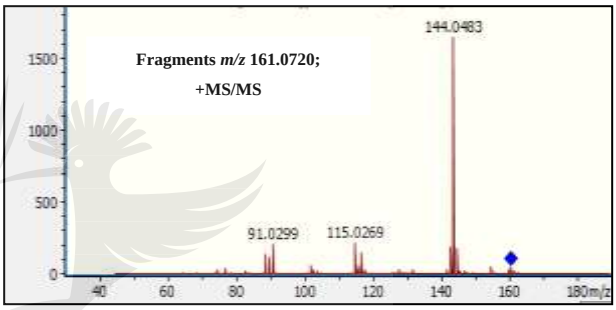
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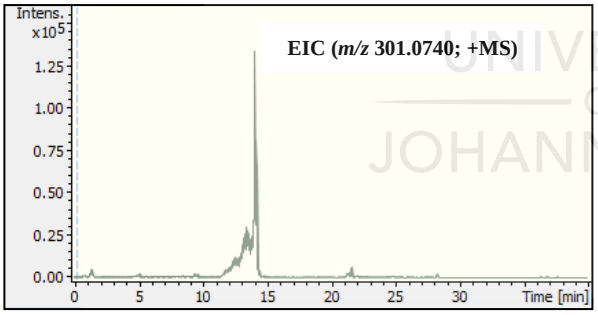	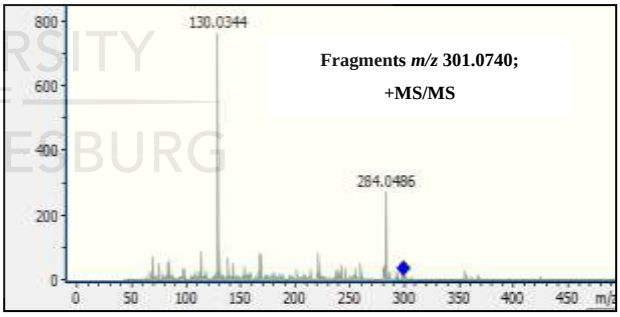
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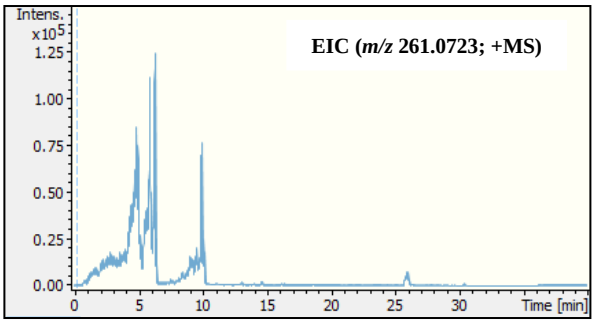	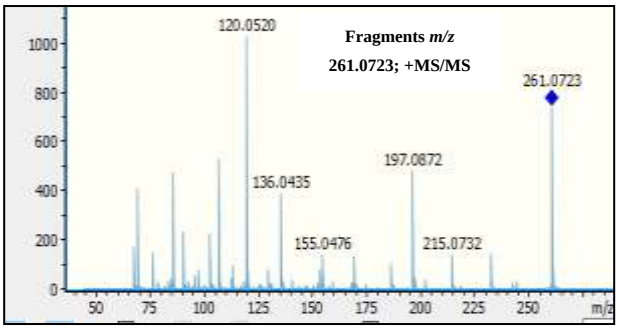

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
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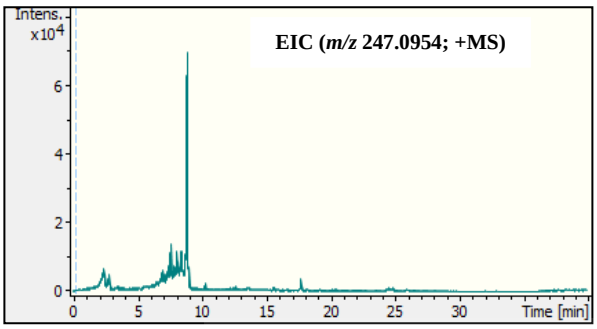	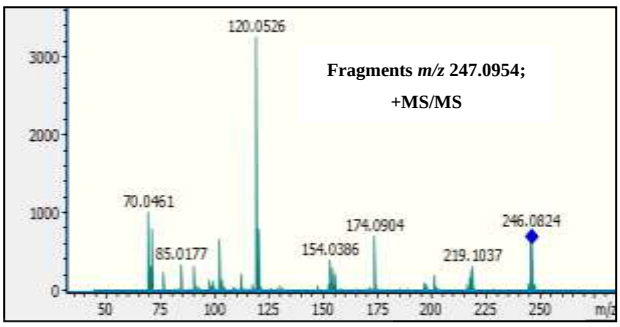
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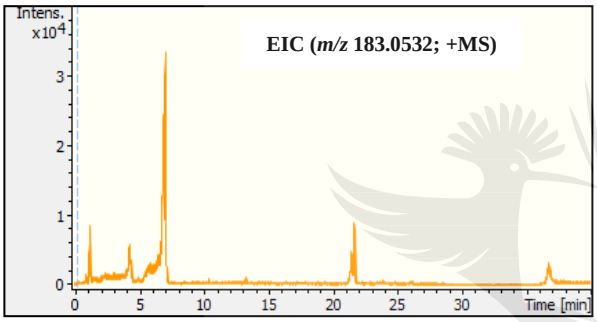	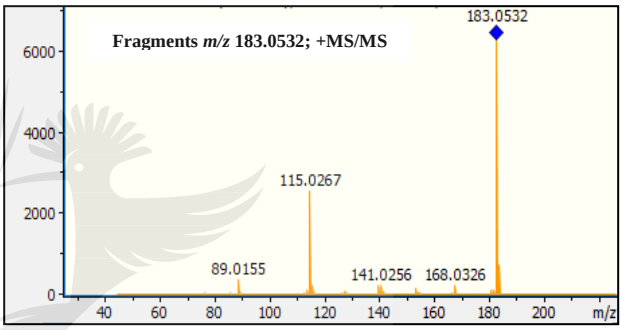
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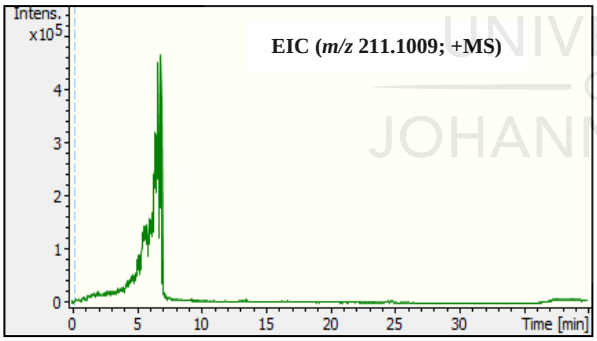	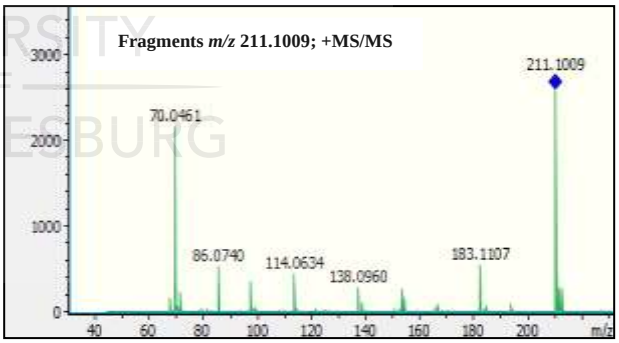
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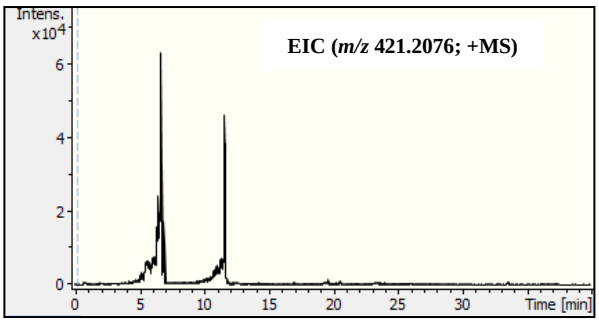	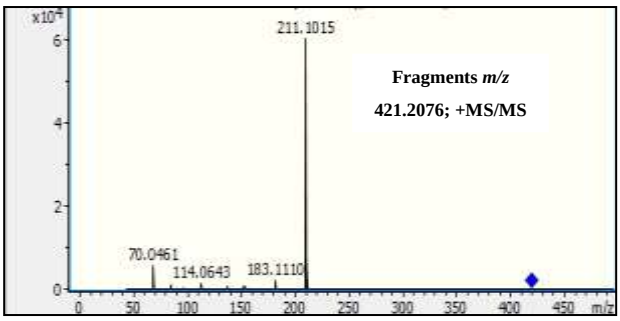

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
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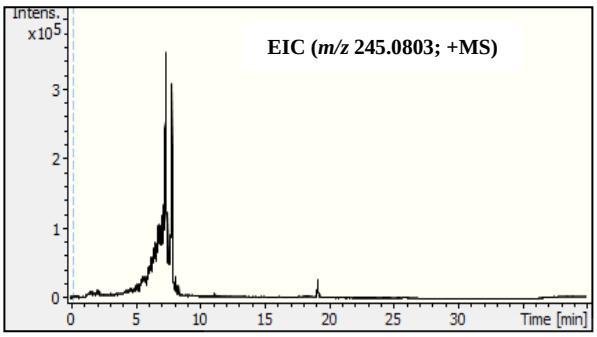	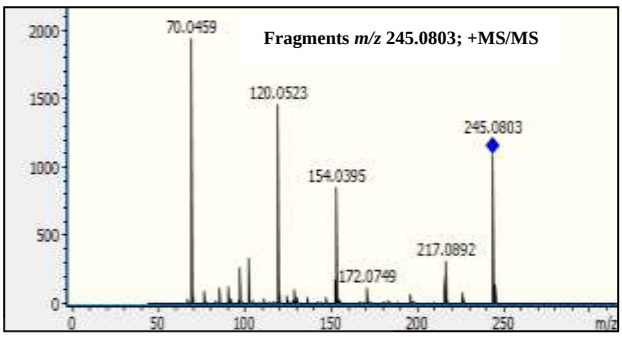
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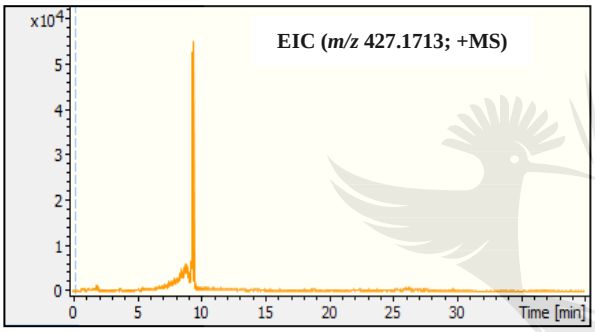	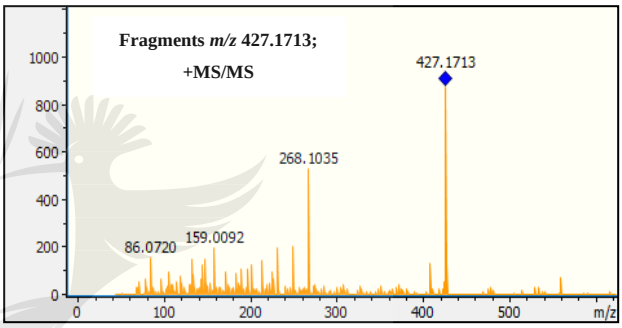
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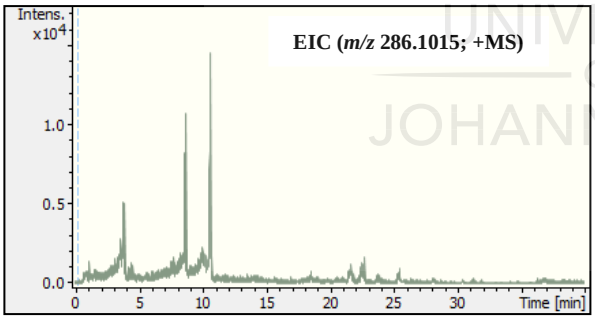	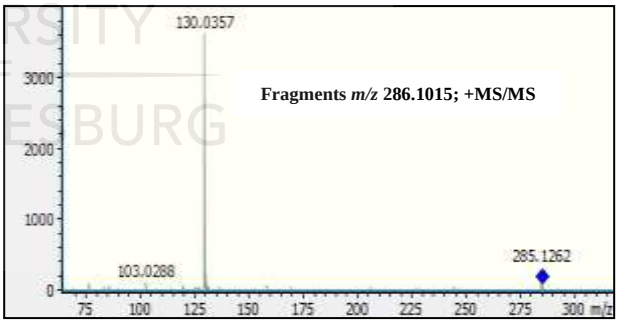
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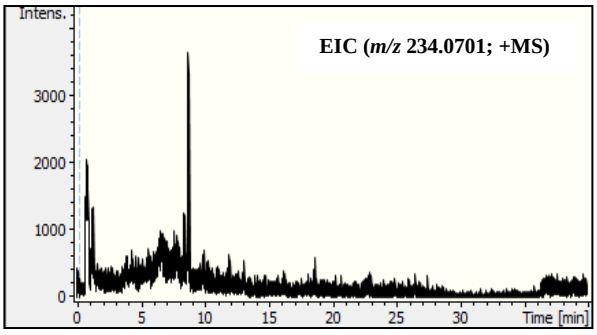	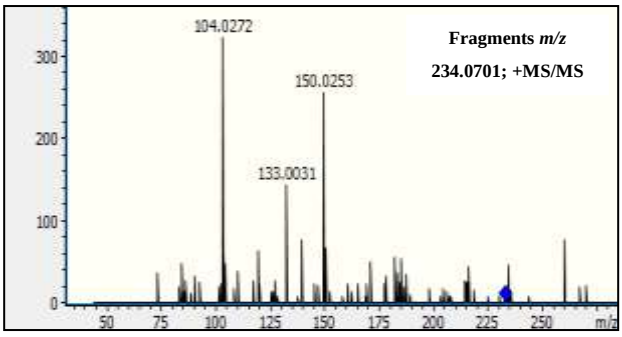

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
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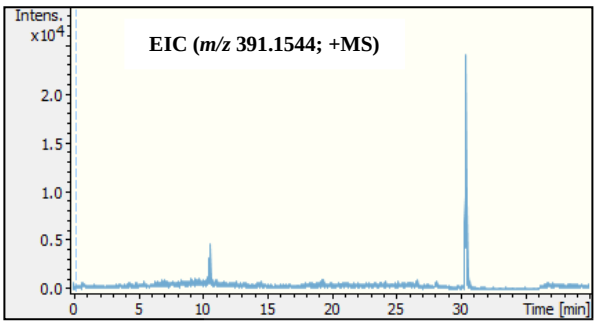	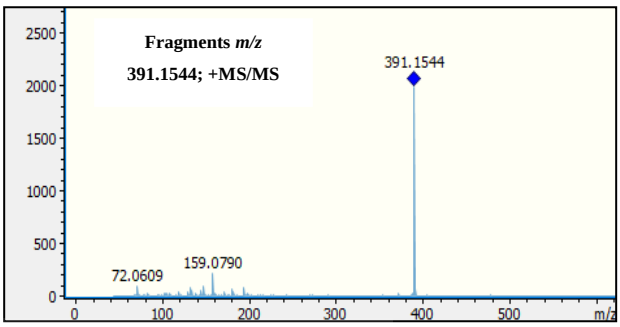
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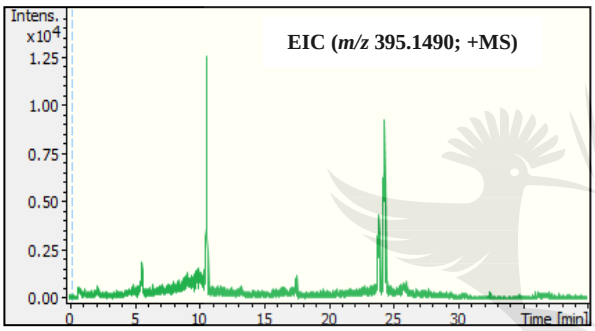	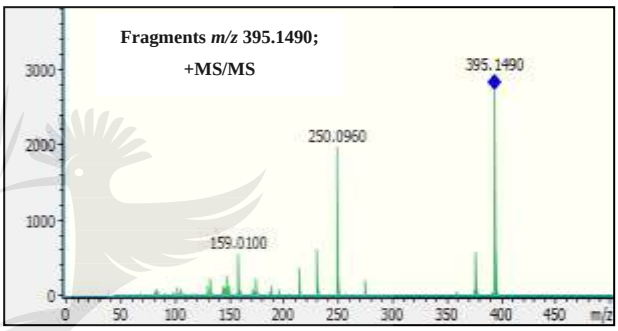
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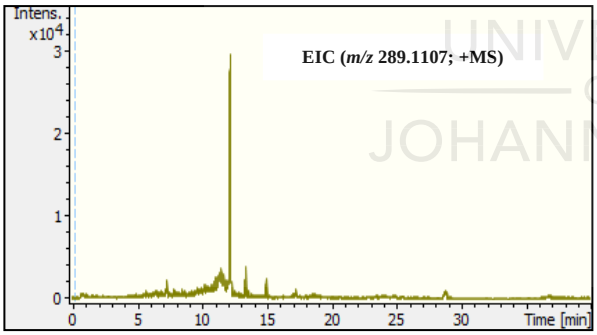	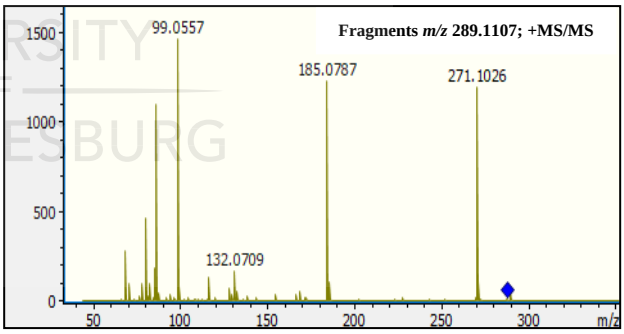
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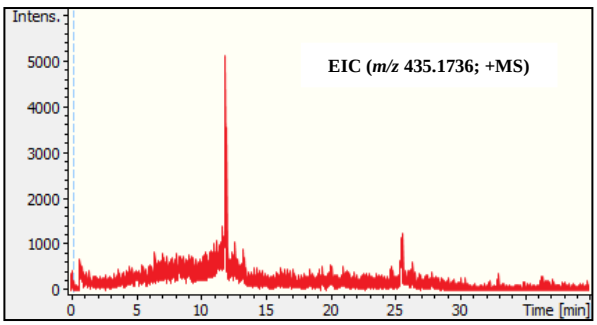
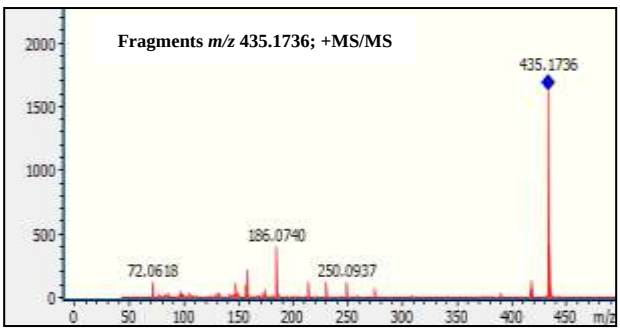
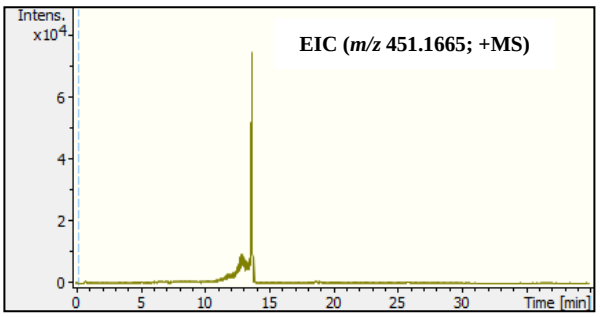
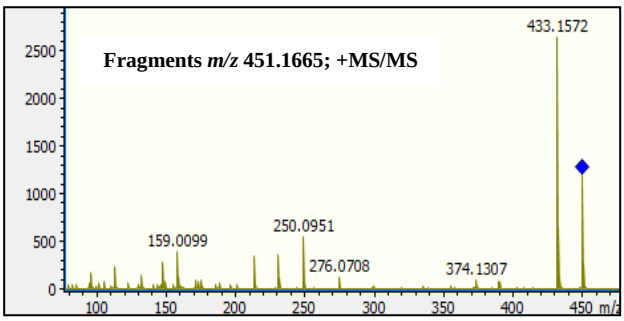
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Auriculoside	
	
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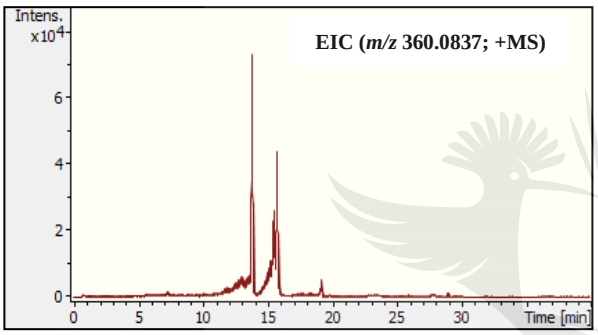	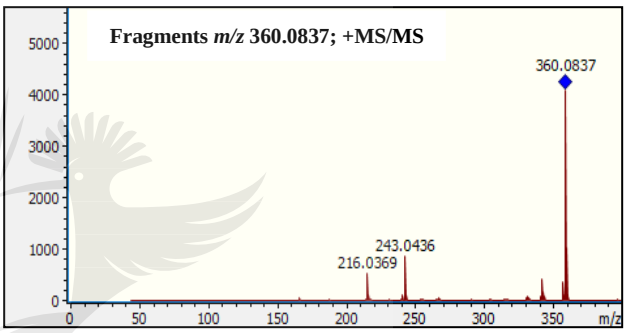
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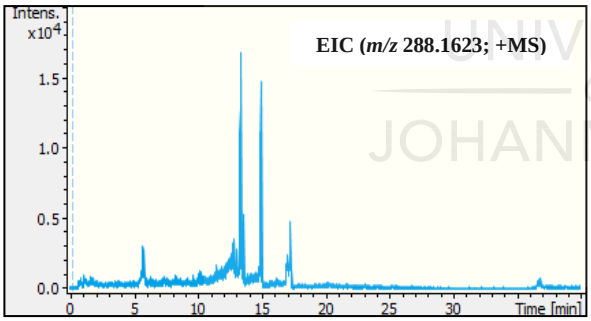	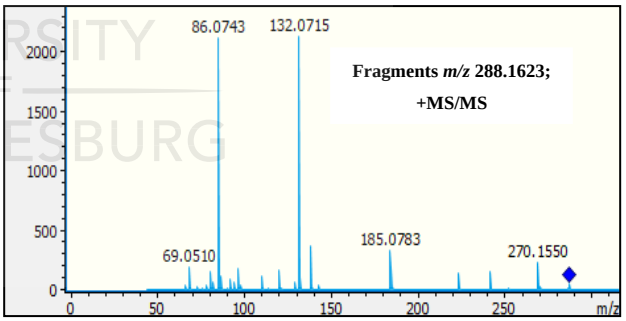
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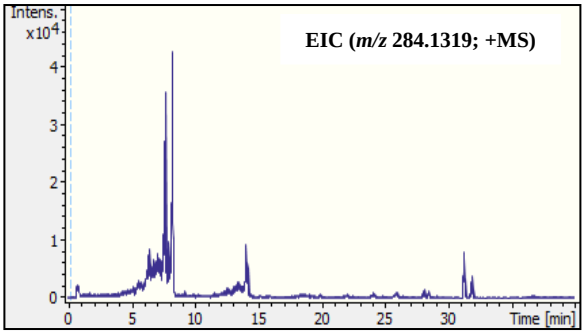
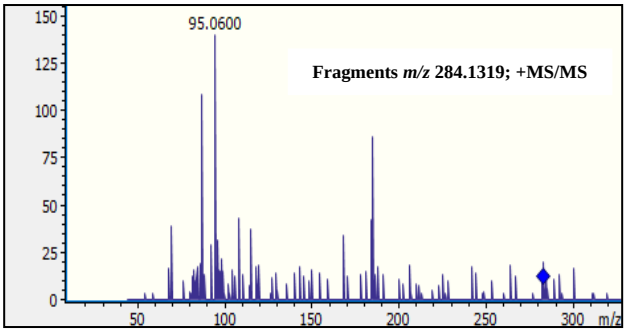
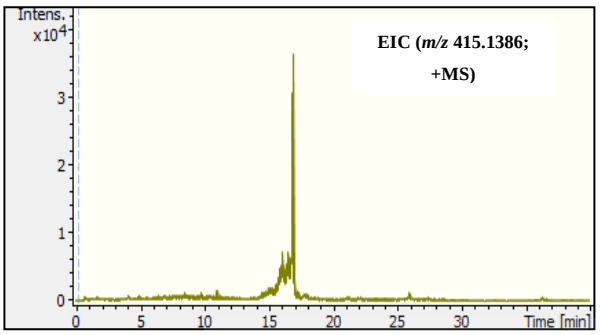
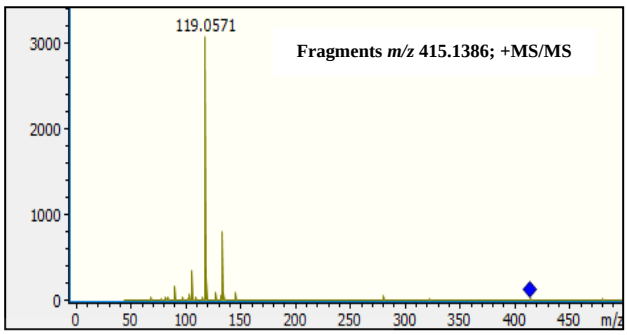
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Picropodophyllin	
	
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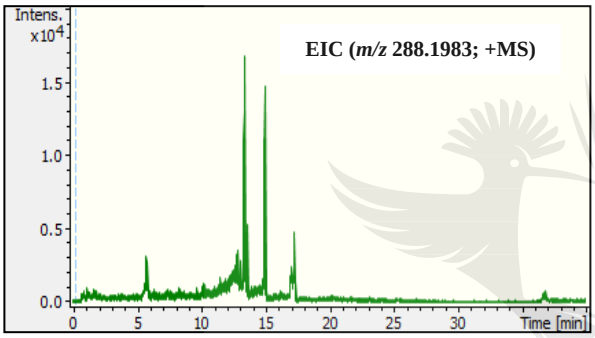
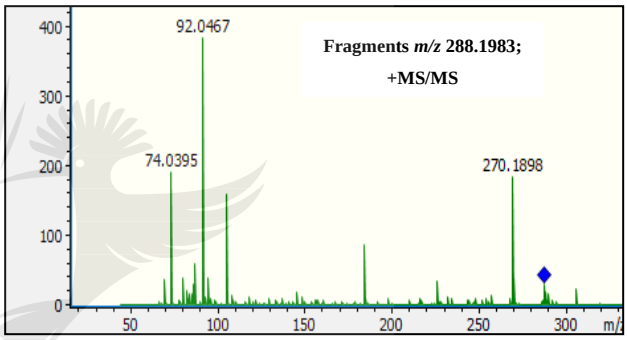
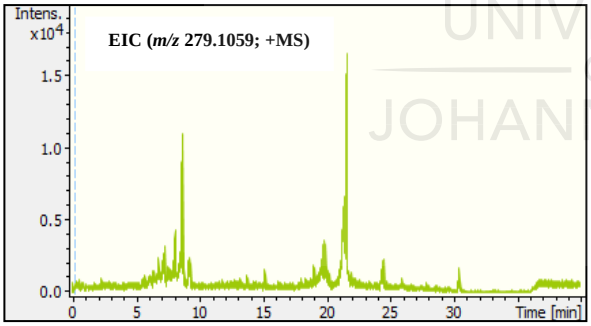
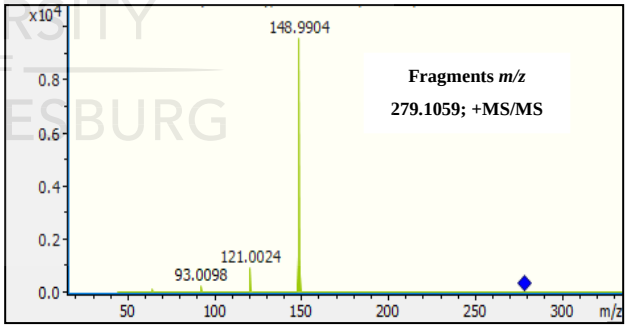
	
Tuliposide A	
	
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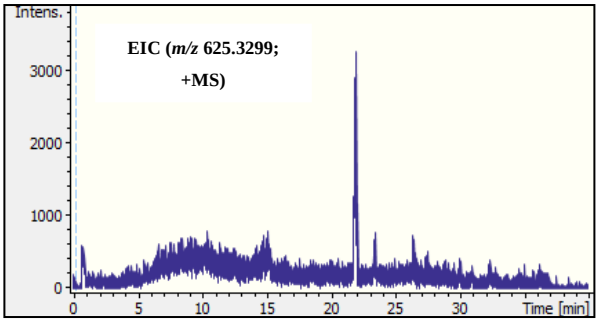
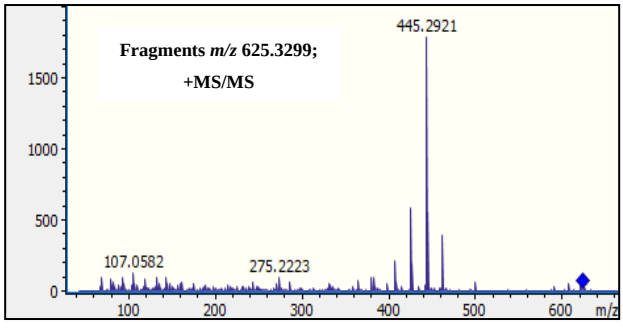
	

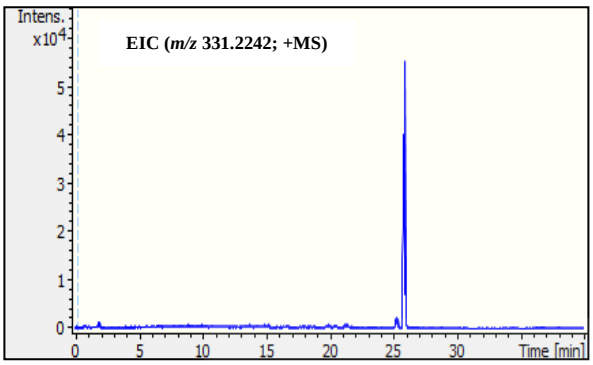
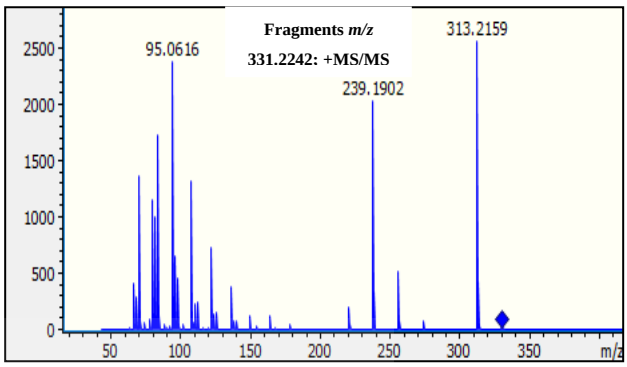
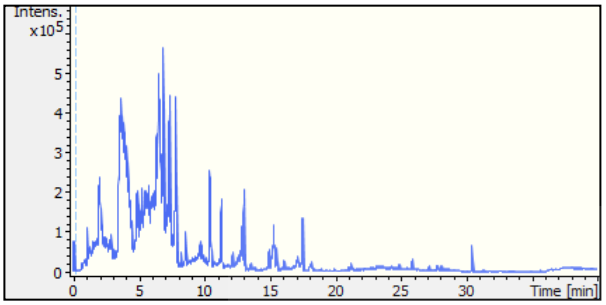
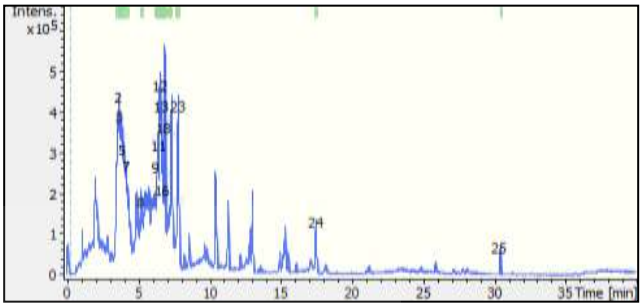
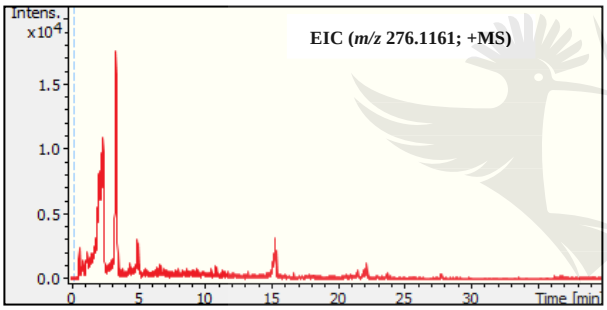
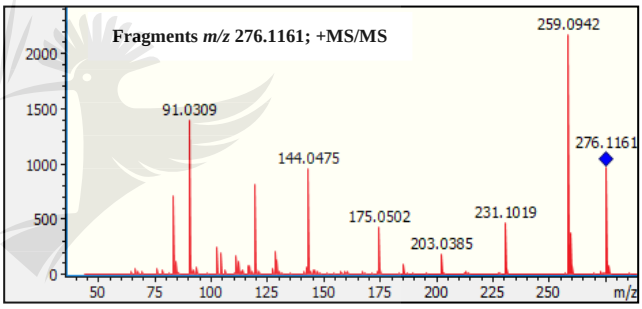
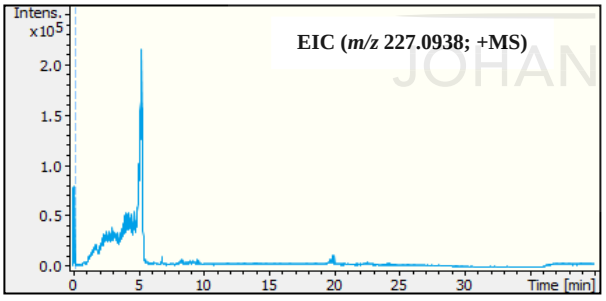
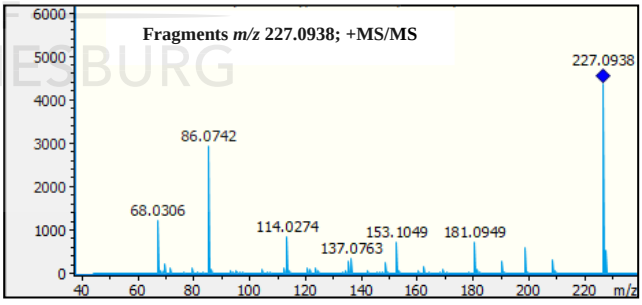
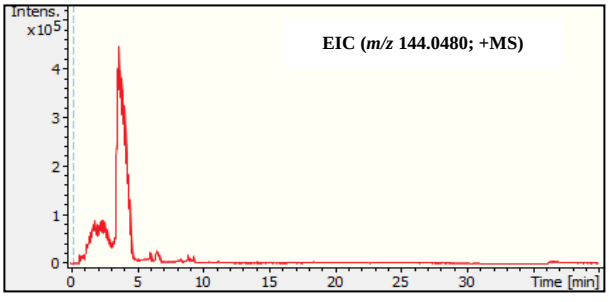
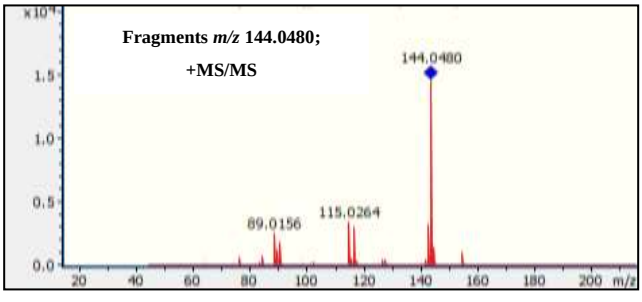
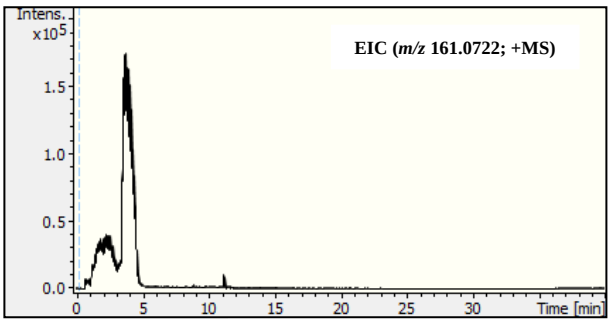
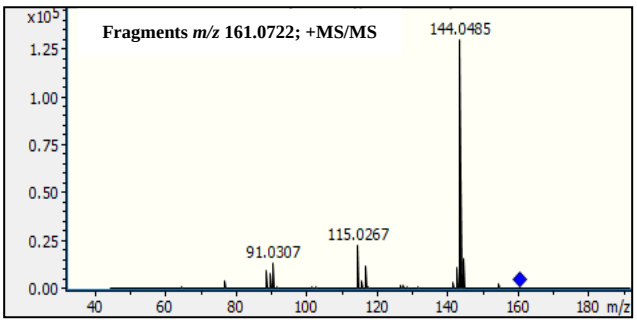
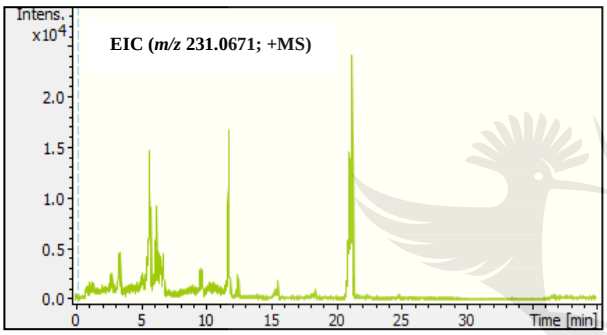
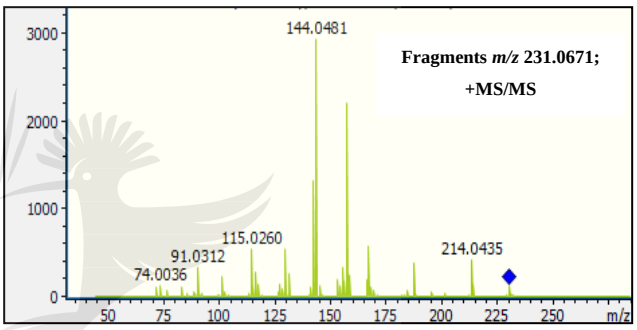
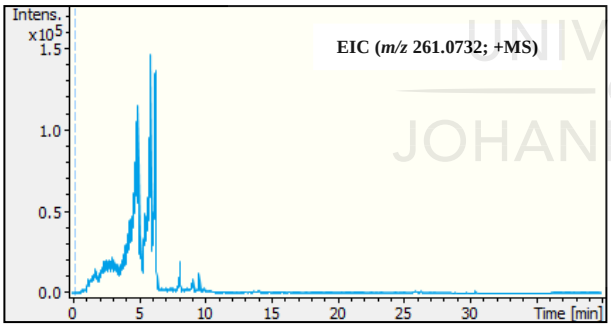
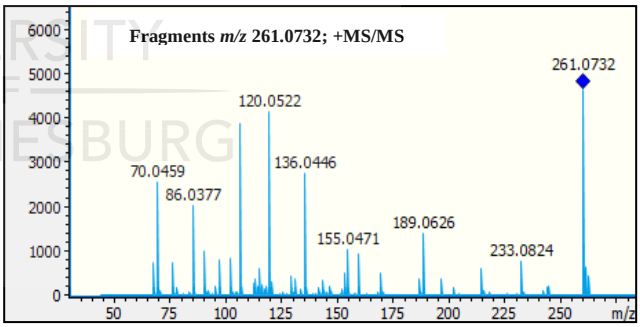
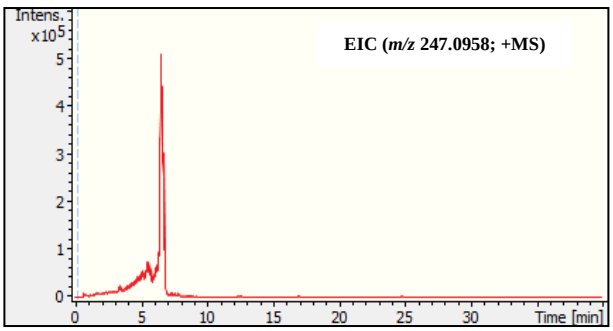
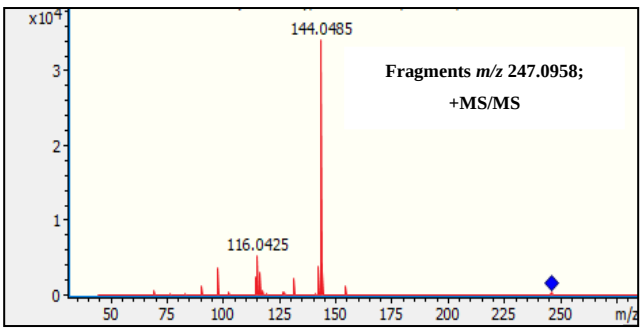
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Cannabielsoin 	

Table A4: Extracted ion chromatogram (EIC) of secondary metabolites from *Bacillus safensis* – NU 04 (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Dubinidine	
	
Genipin	
	
4-Methyl-5-(2'-hydroxyethyl)-thiazole	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Anatabine	
	
Visnagin	
	
Khellin	
	
(R)-Columbianetin	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
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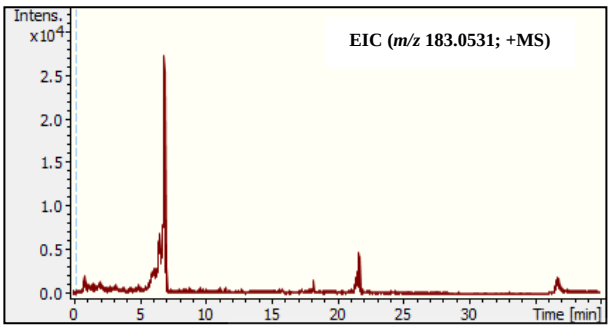
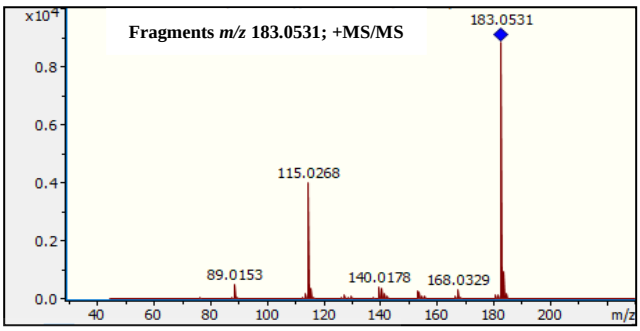
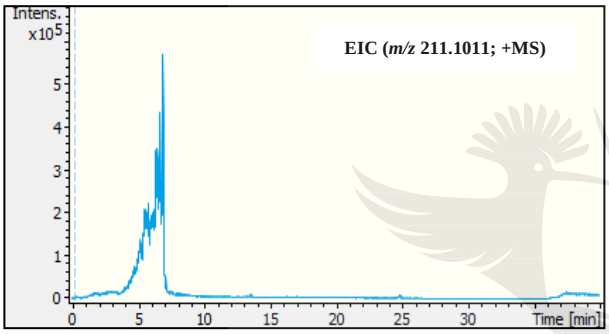
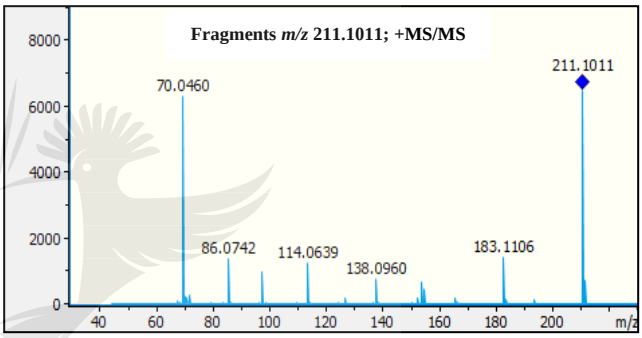
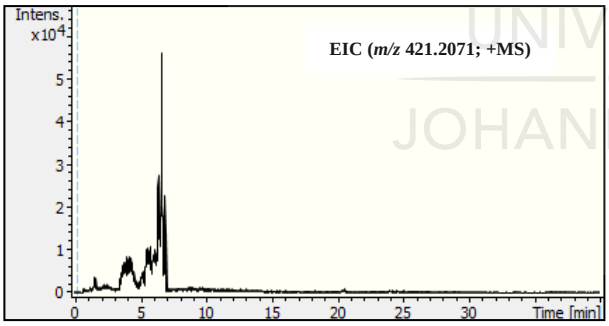
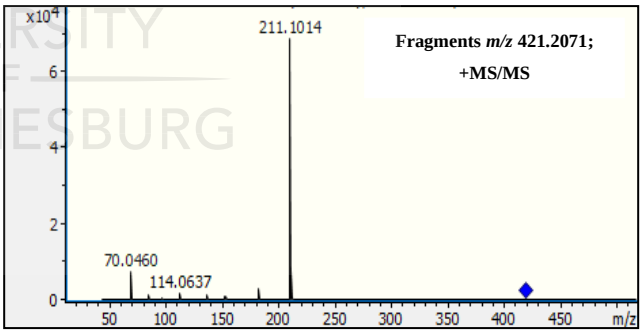
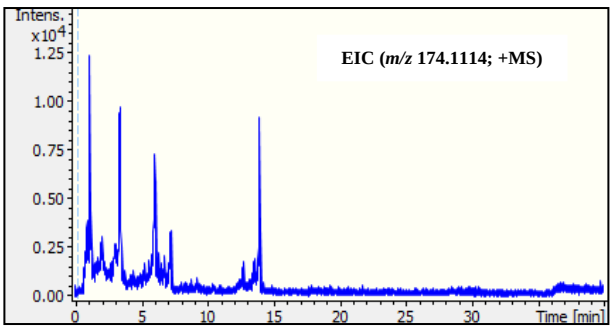
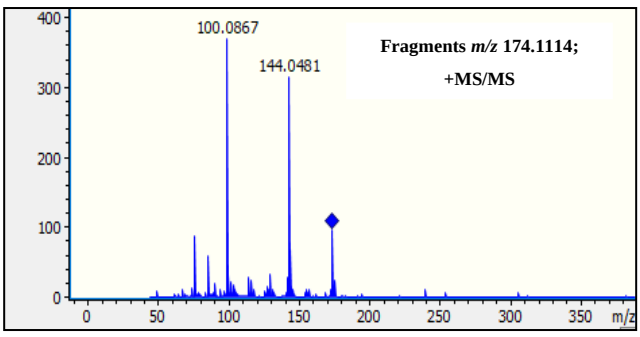
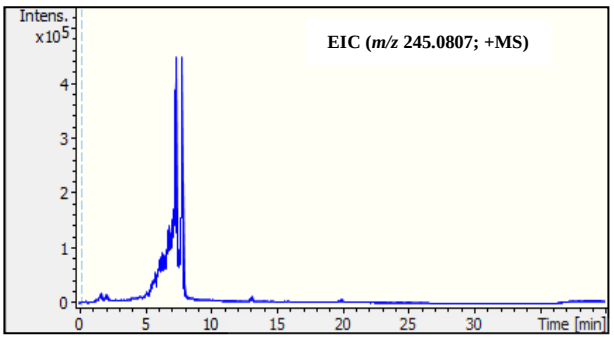
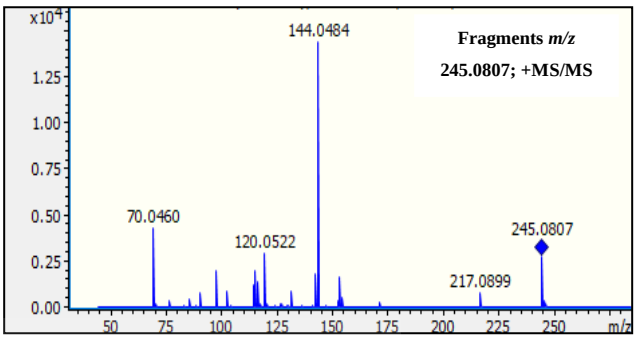
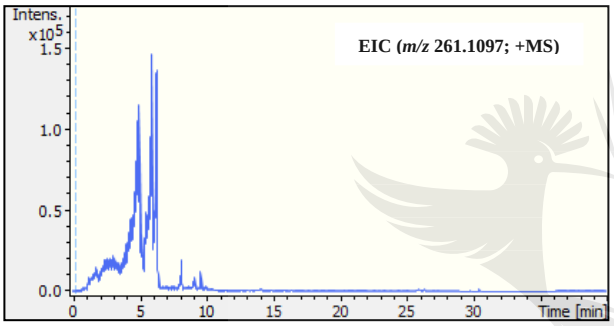
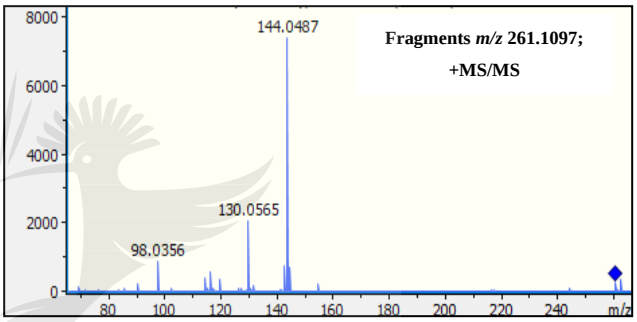
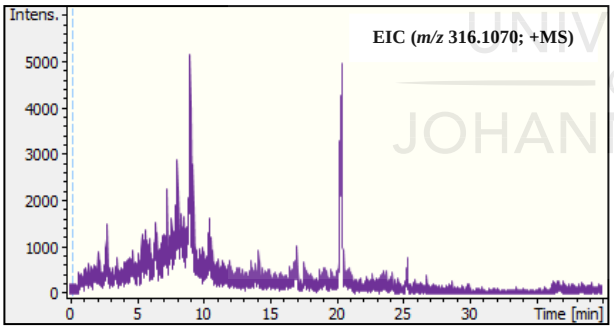
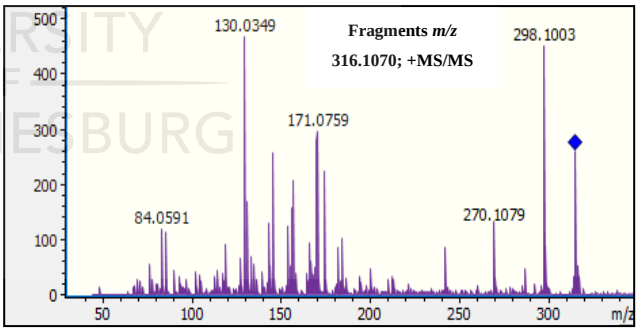
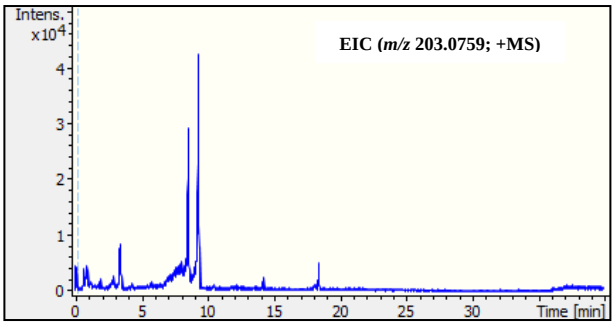
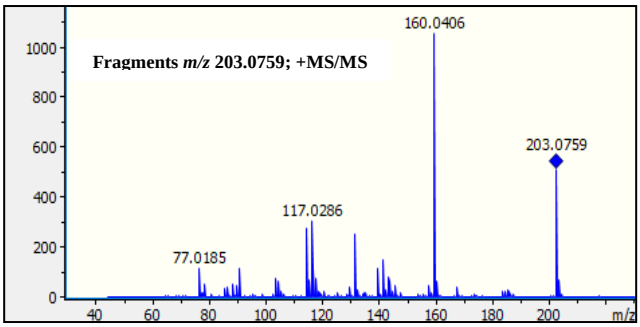
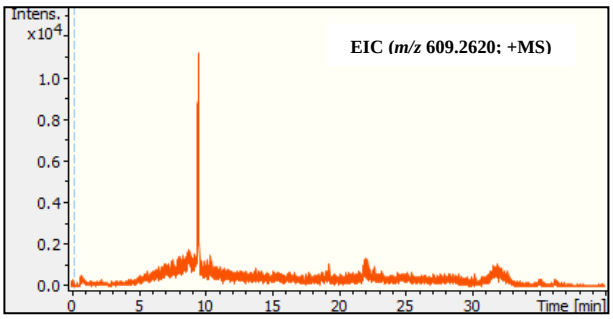
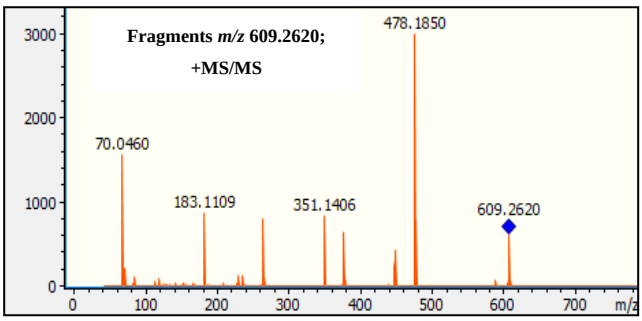
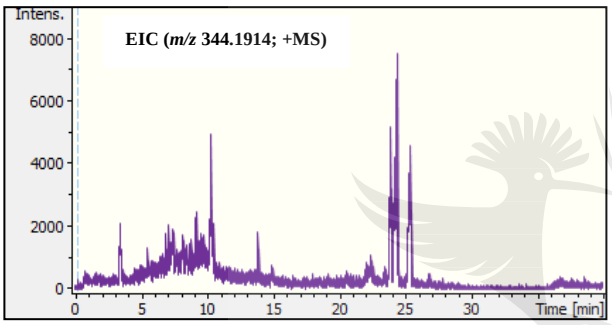
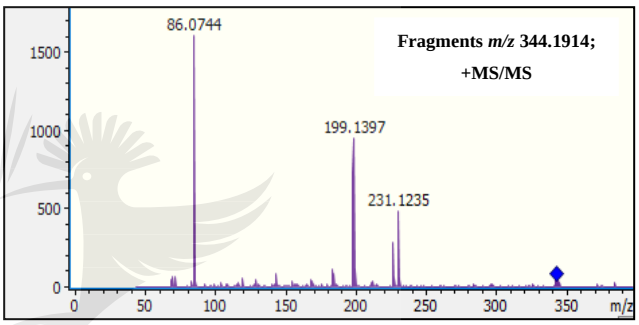
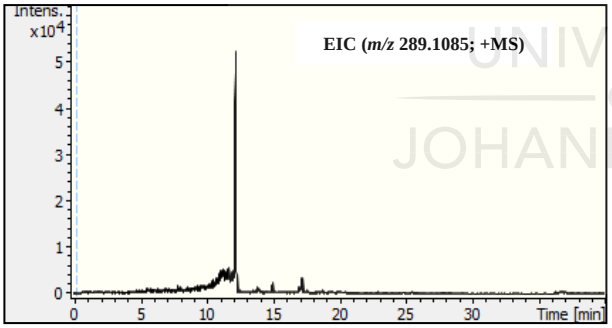
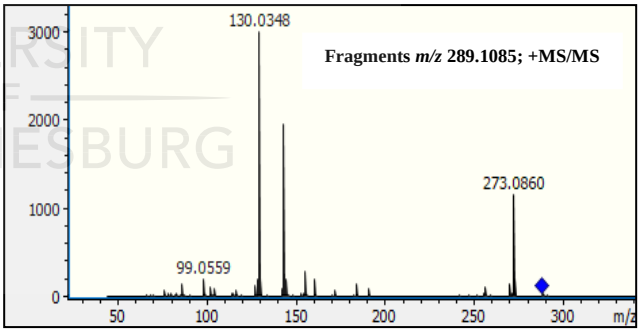
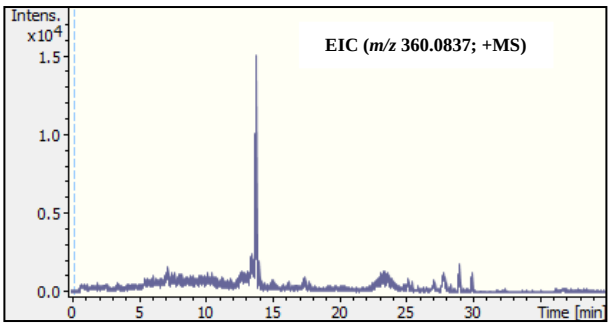
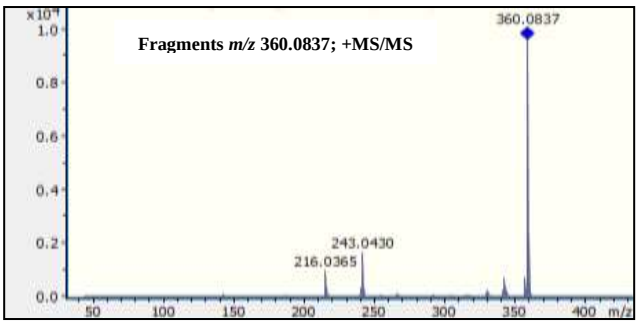
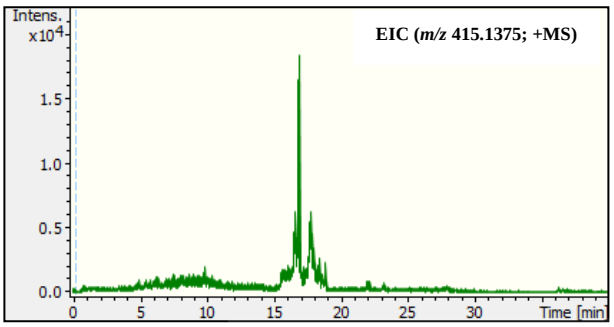
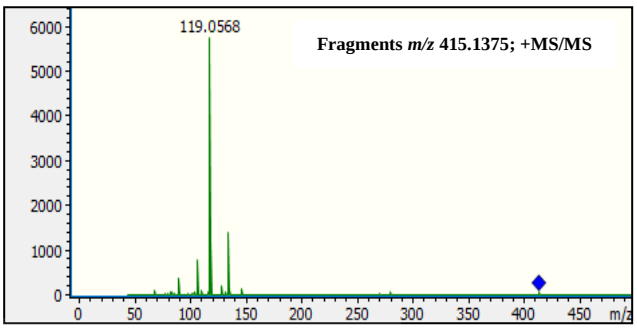
	
Sinapyl alcohol	
	
Eupaformosanin	
	
Swainsonine	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Fulvoplumierin	
	
8-Deoxylactucin	
	
Rosinidin	
	
Vasicinone	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Reserpine	
	
(-)-Laudanidine	
	
Asebogenin	
	
Triglochinin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Picropodophyllin	
	
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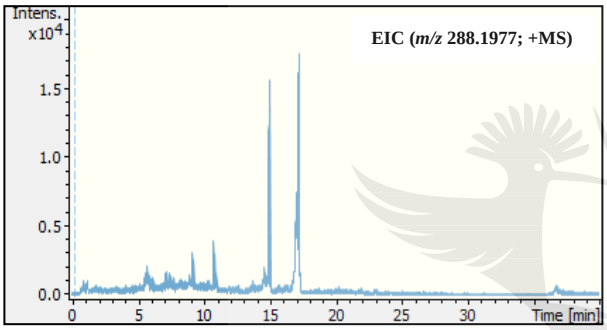
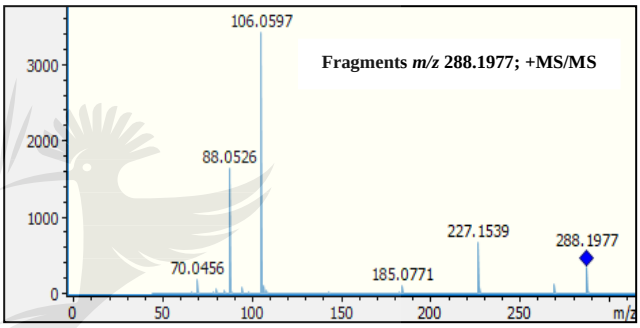
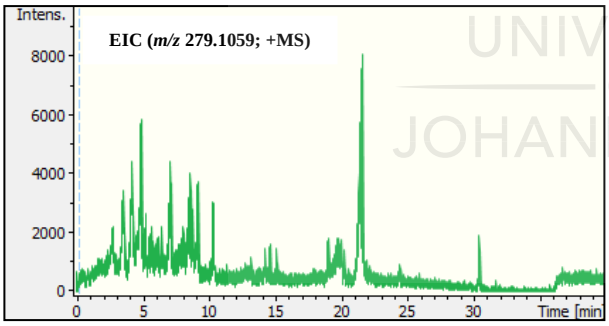
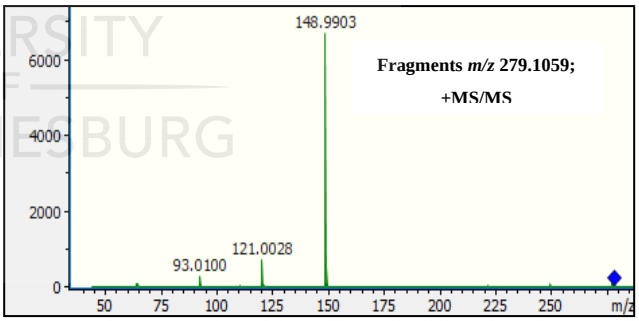
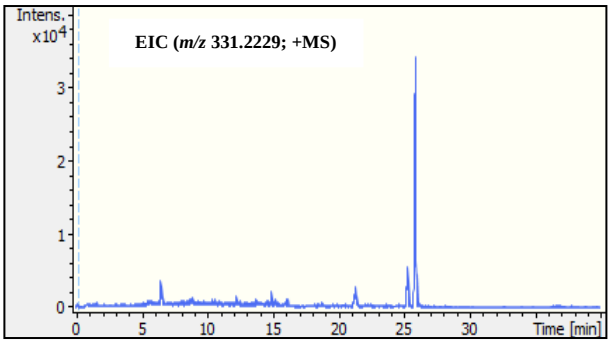
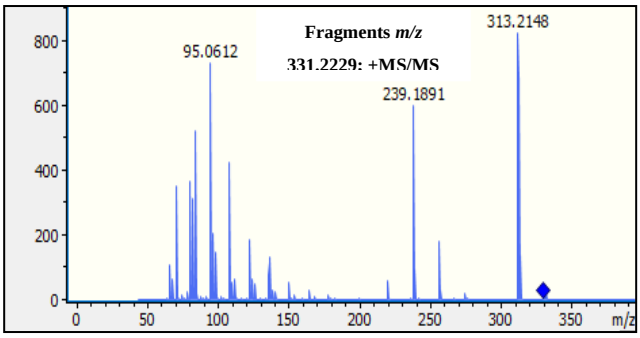
	
Tuliposide A	
	
Cannabielsoin	
	

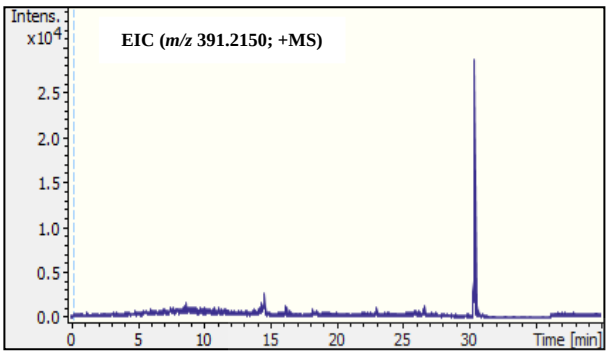
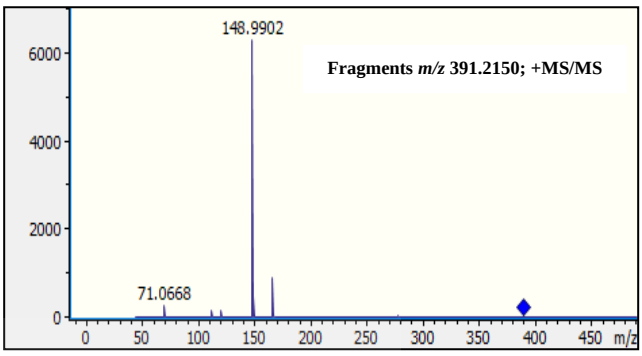
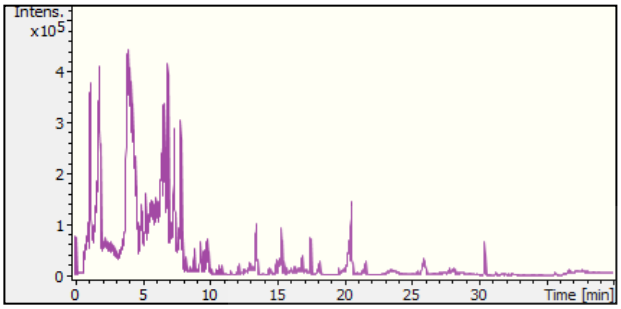
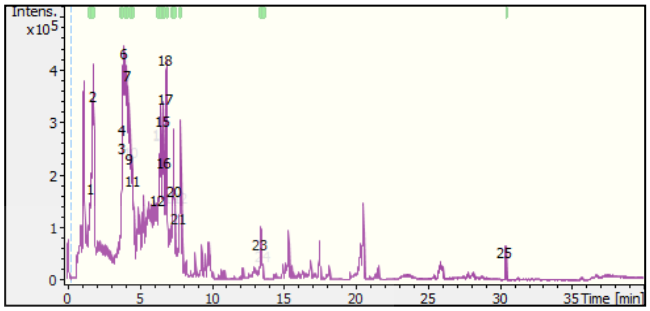
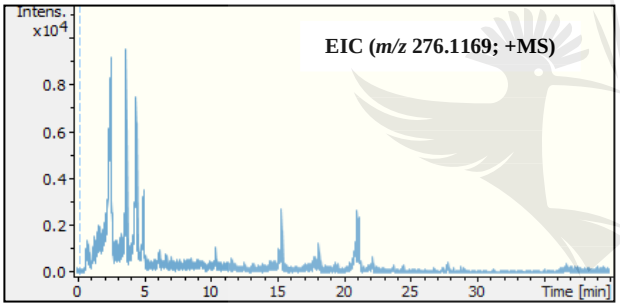
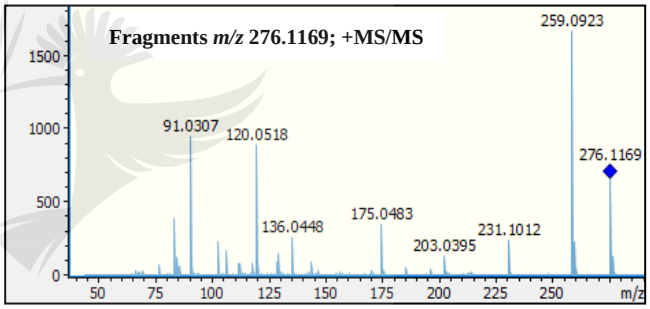
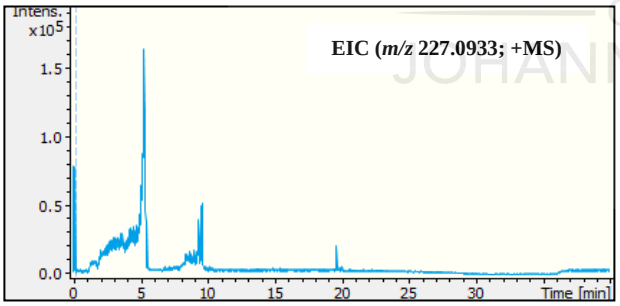
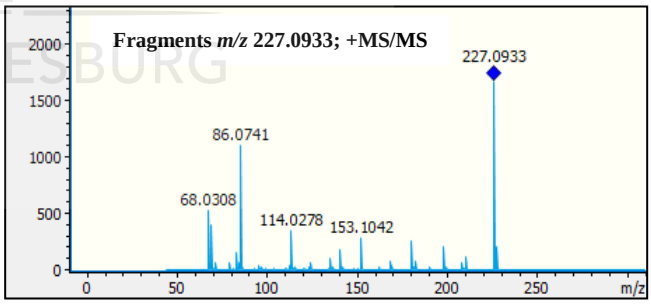
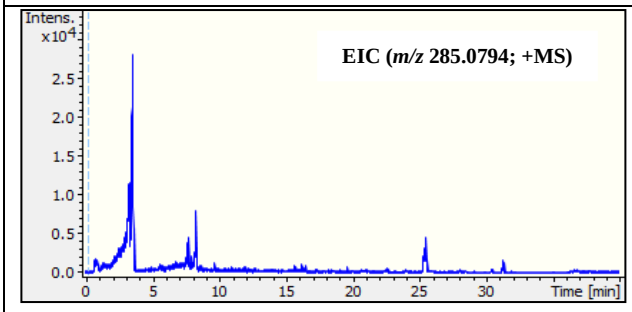
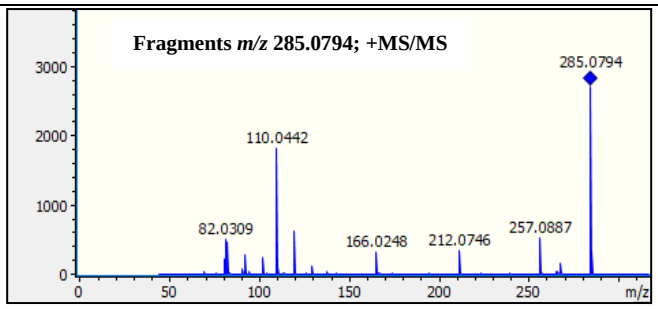
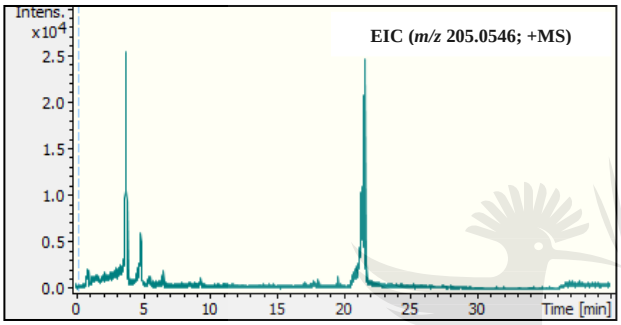
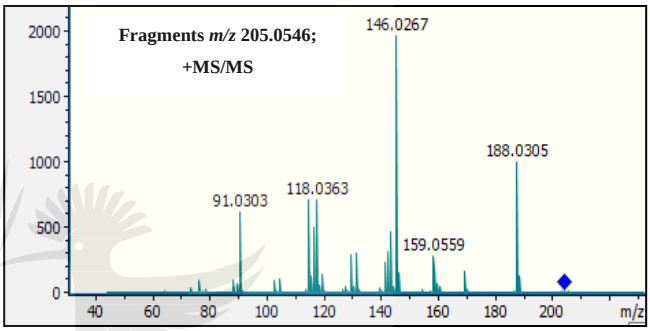
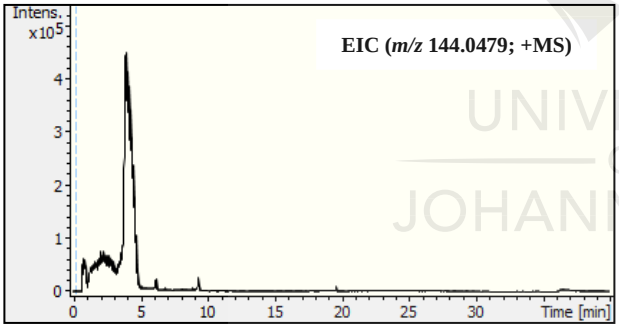
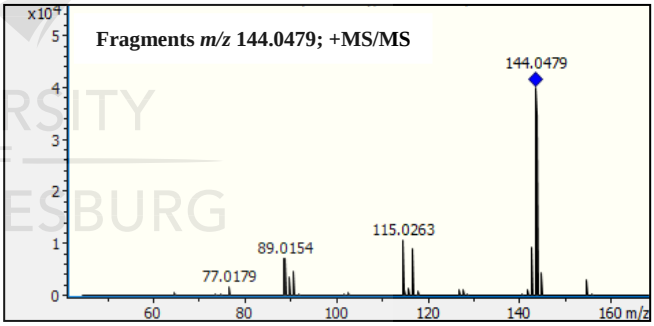
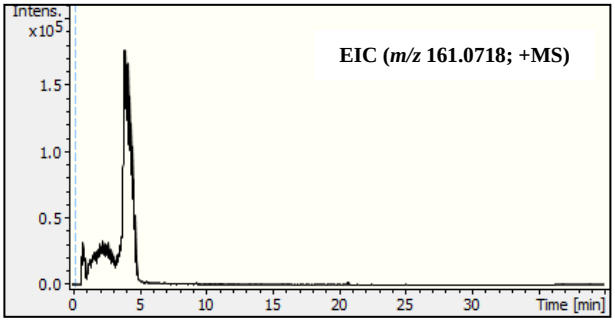
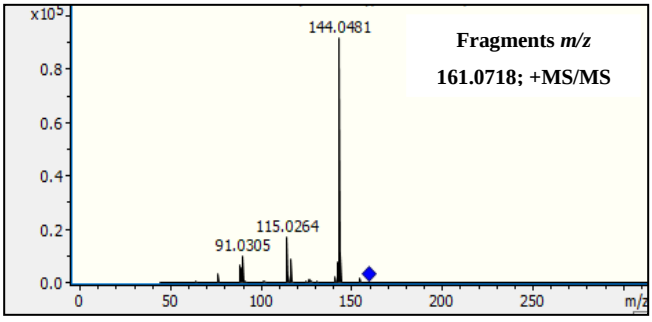
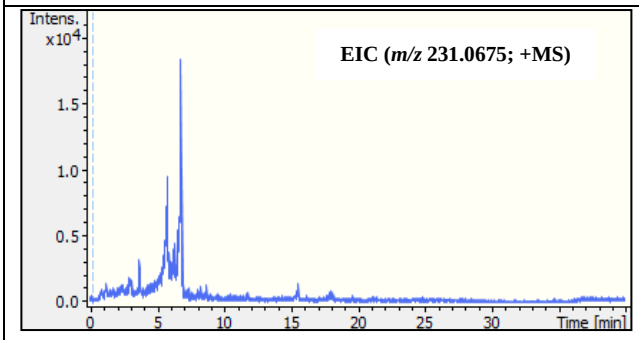
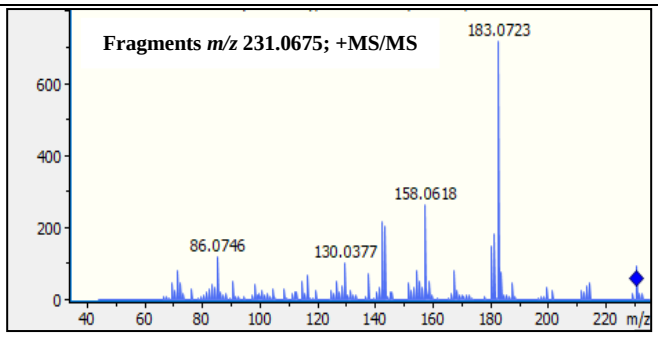
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Neoquassin 	

Table A5: Extracted ion chromatogram (EIC) of secondary metabolites from *Pantoea vagans* – NU 05 (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Dubinidine	
	
Genipin	
	
Xanthosine	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
	
Droserone	
	
4-Methyl-5-(2'-hydroxyethyl)-thiazole	
	
Anatabine	
	
Visnagin	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
	
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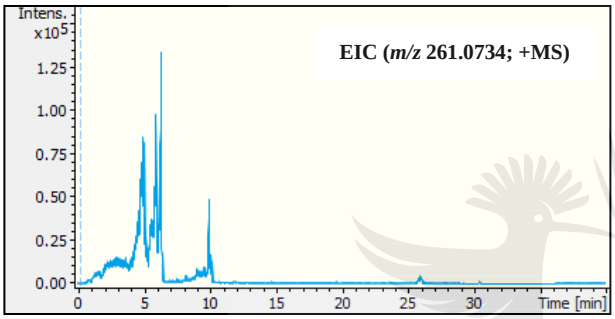	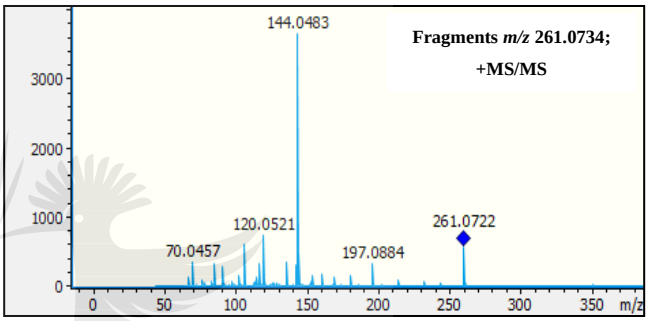
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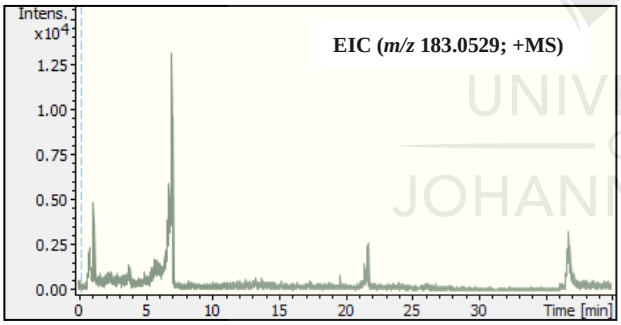
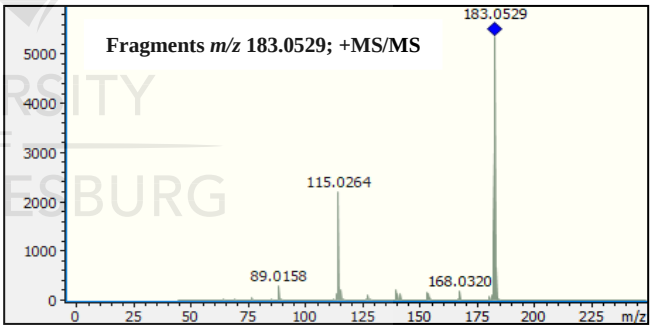
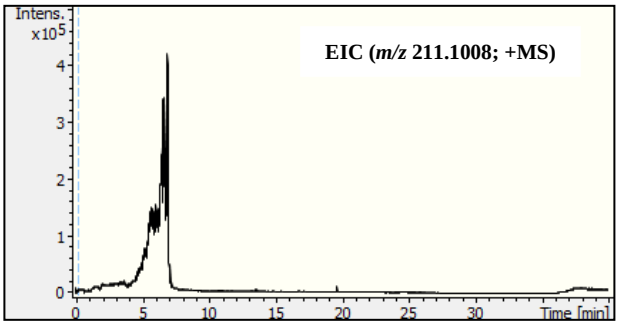
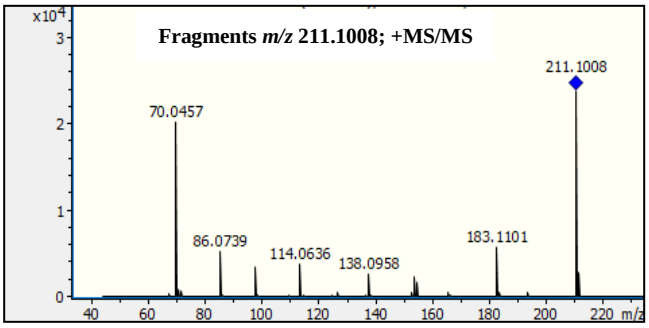
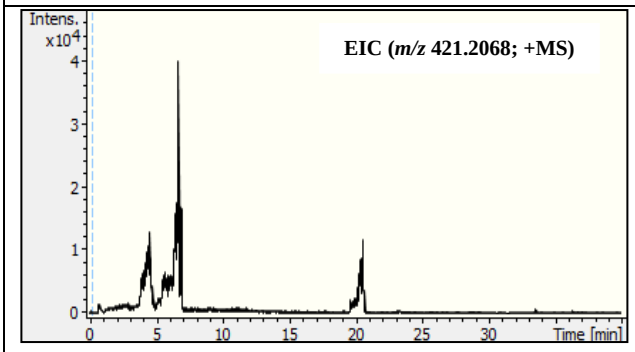
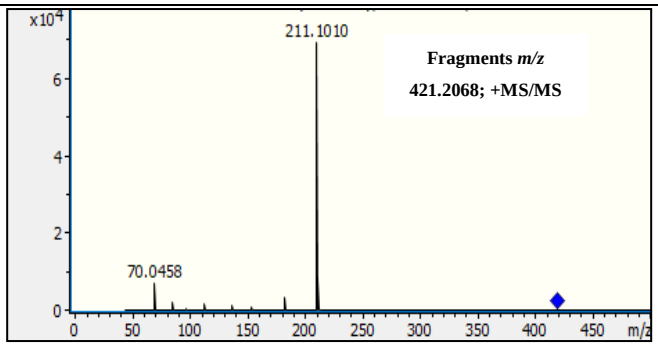
	
Sinapyl alcohol	
	
Eupaformosanin	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
	
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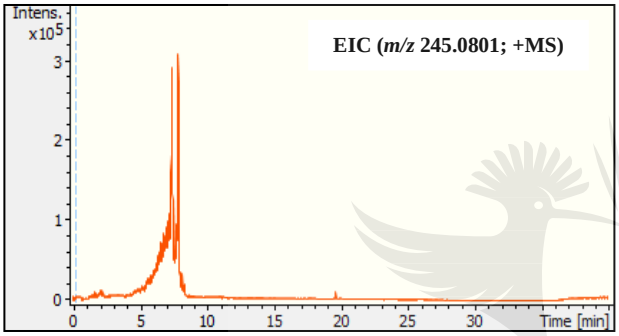	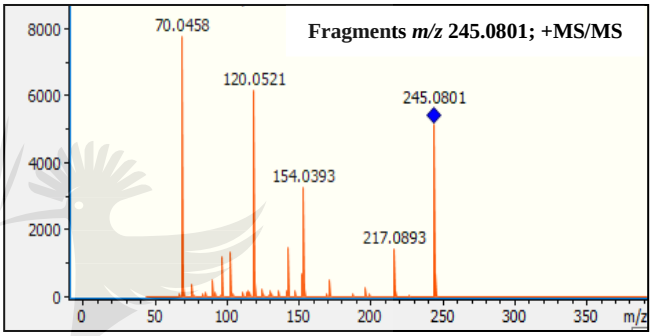
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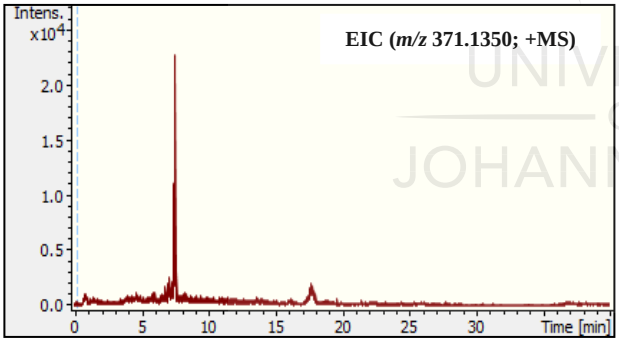
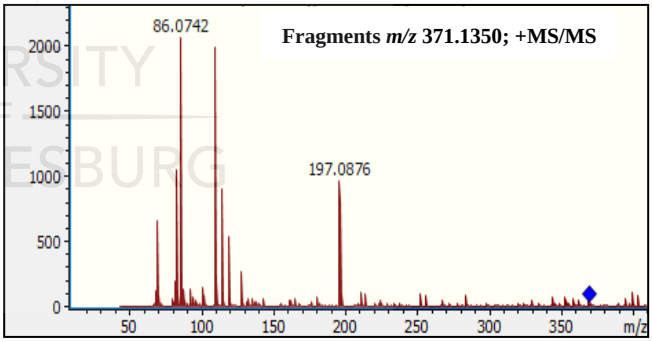
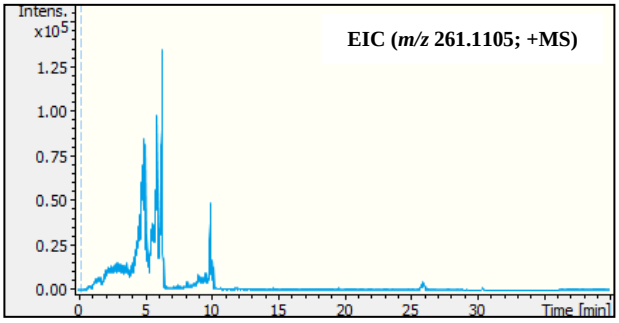
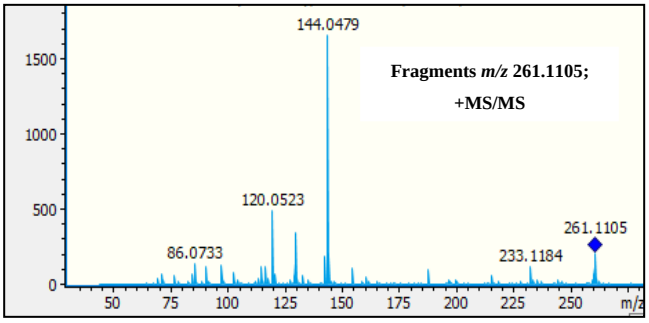
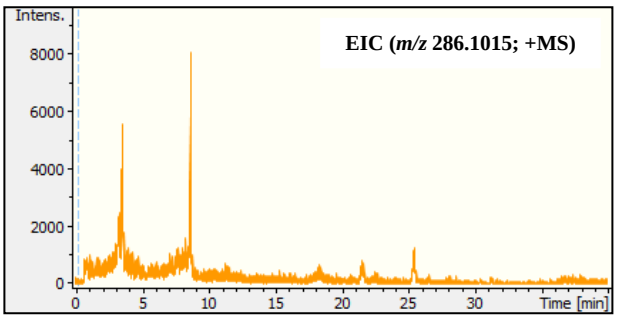
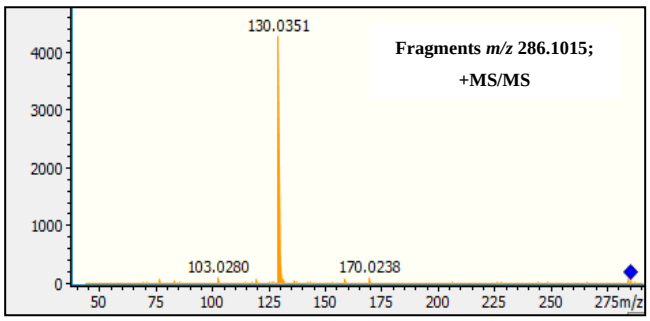
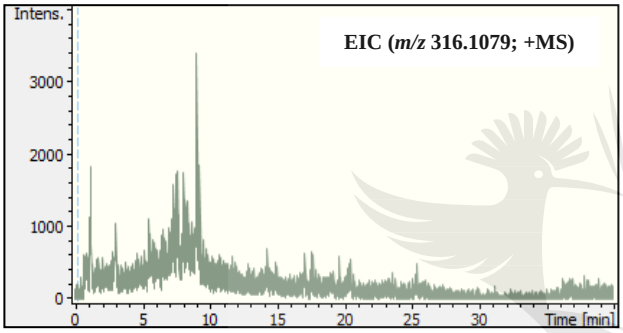
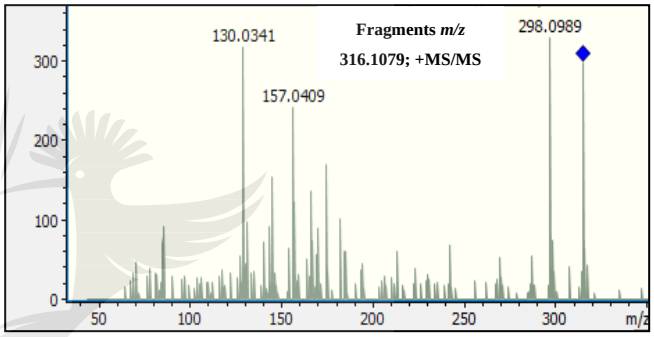
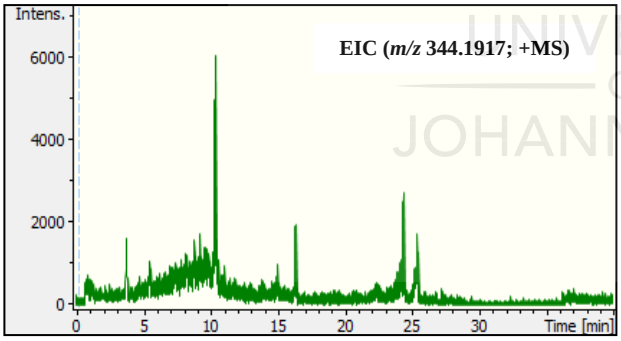
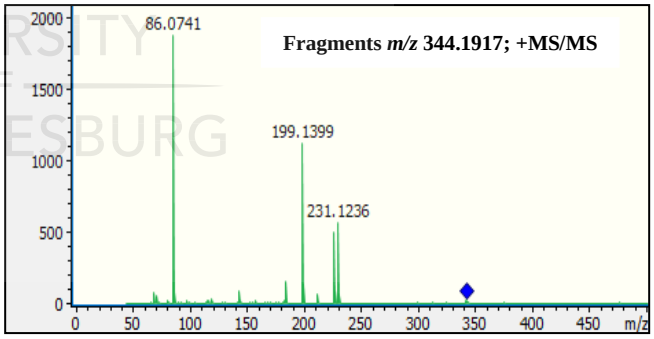
	
8-Deoxylactucin	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Arborinine	
	
Rosinidin	
	
(-)-Laudanidine	
	
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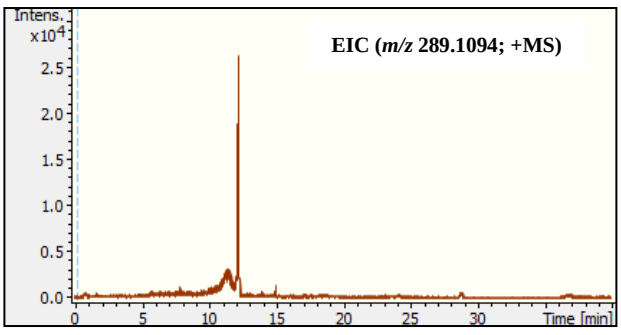
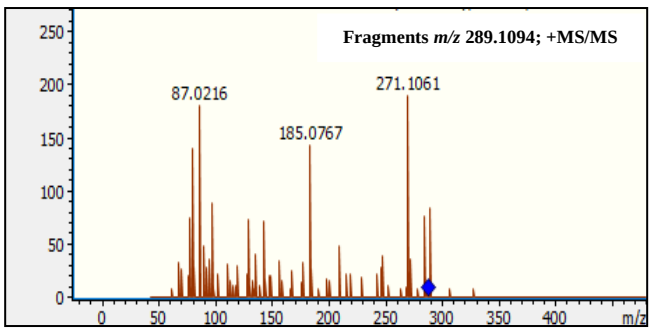
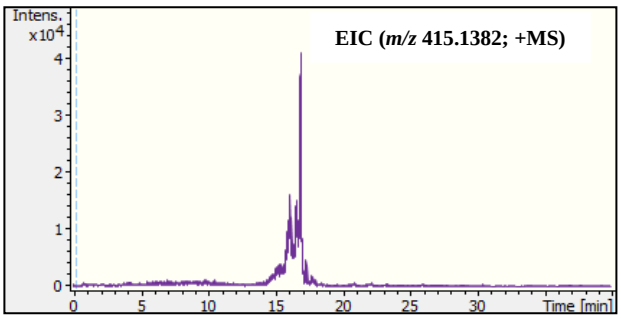
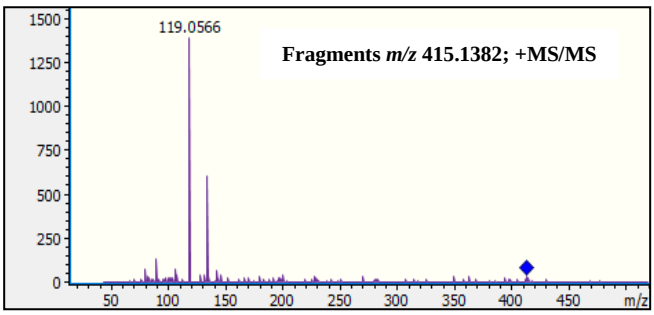
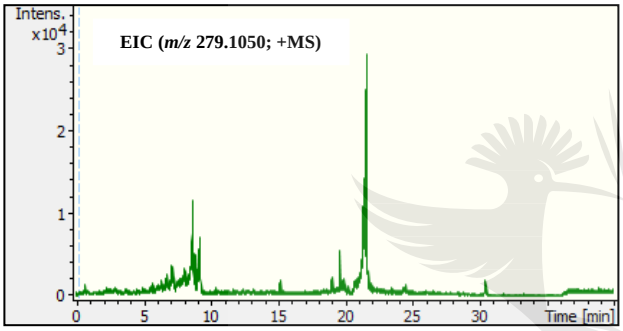
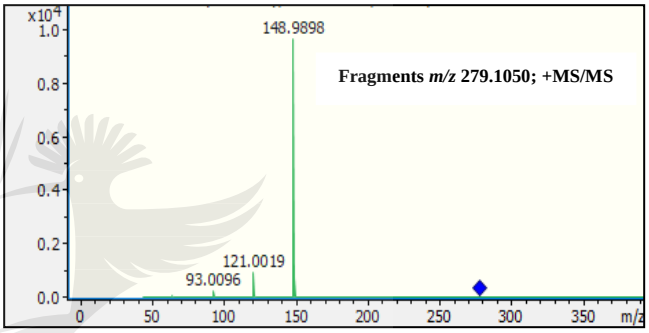
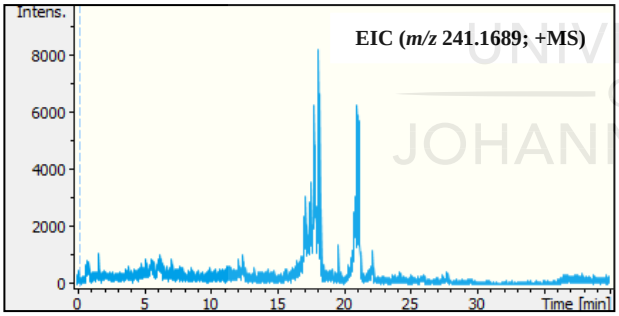
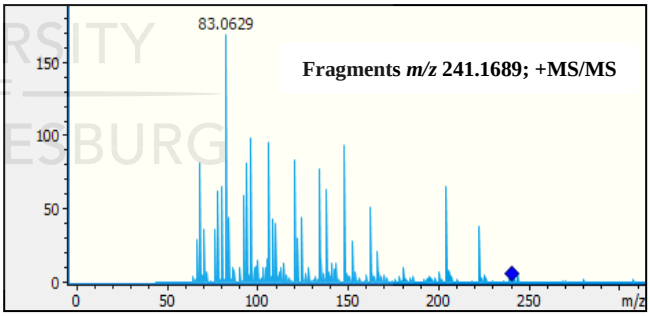
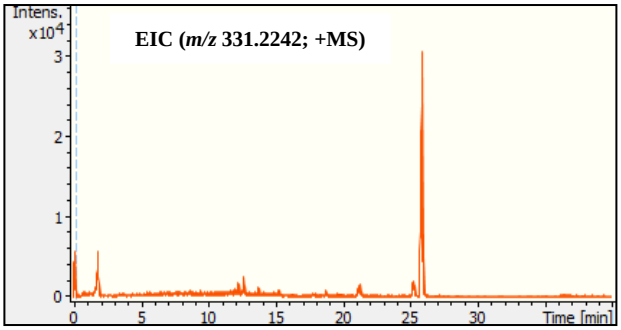
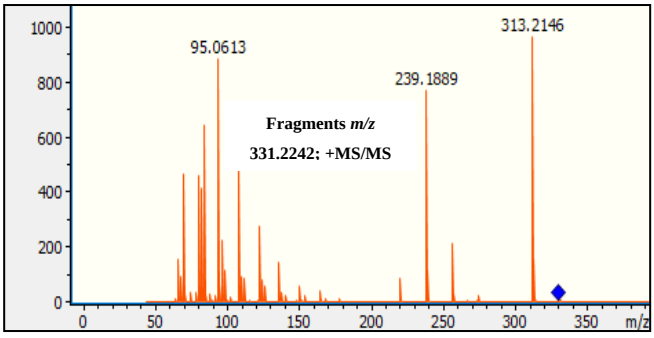
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Picropodophyllin	
	
Tuliposide A	
	
Borrerine	
	
Cannabielsoin	
	

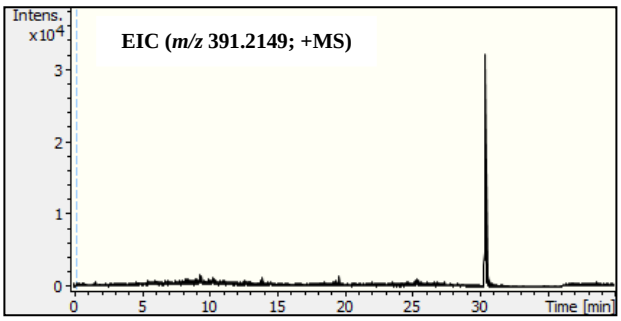
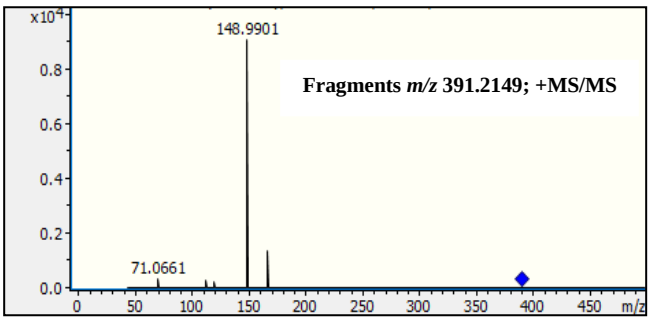
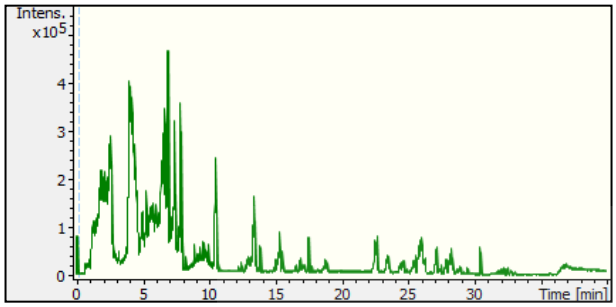
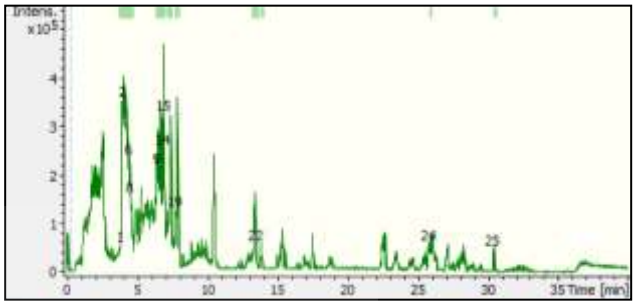
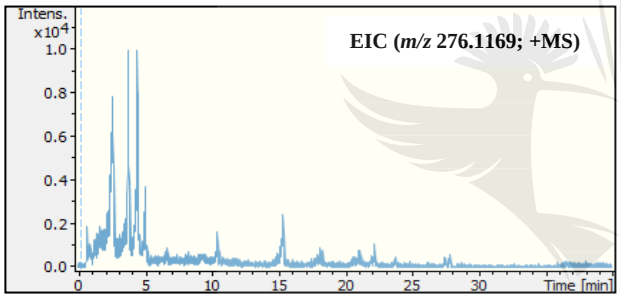
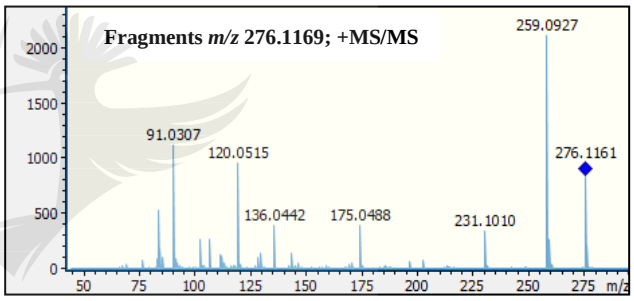
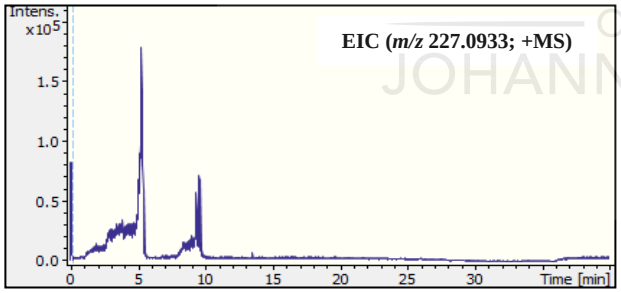
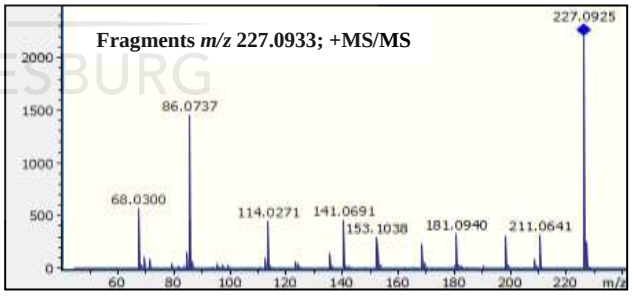
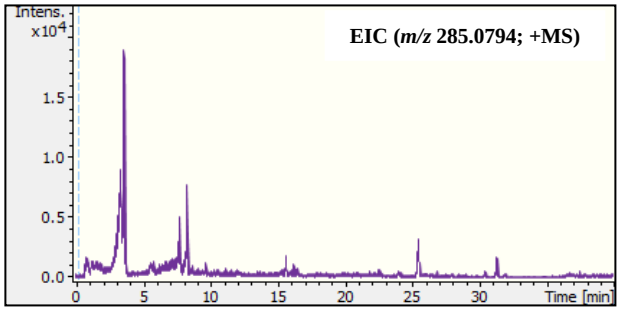
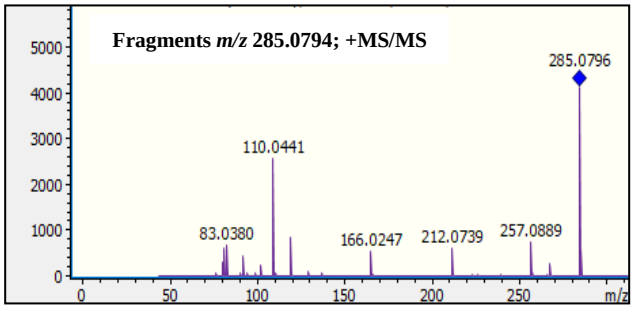
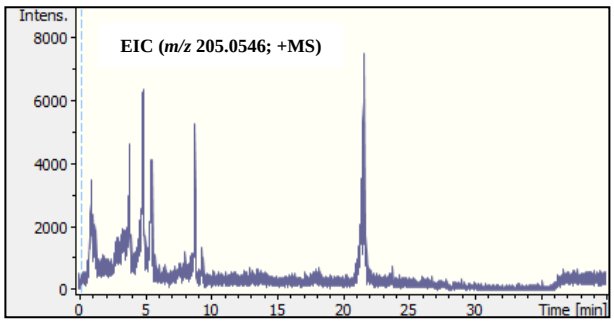
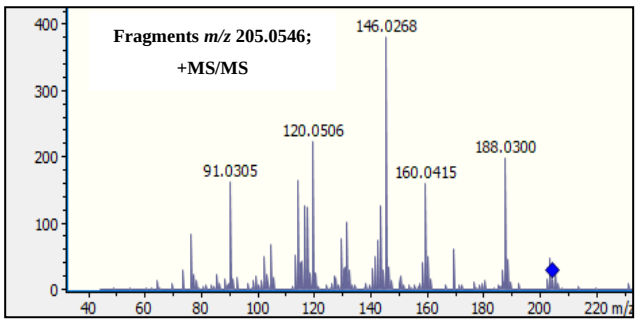
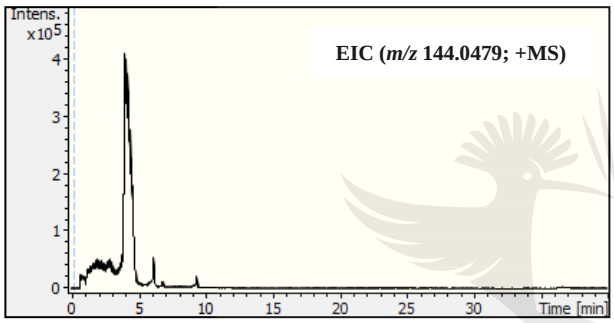
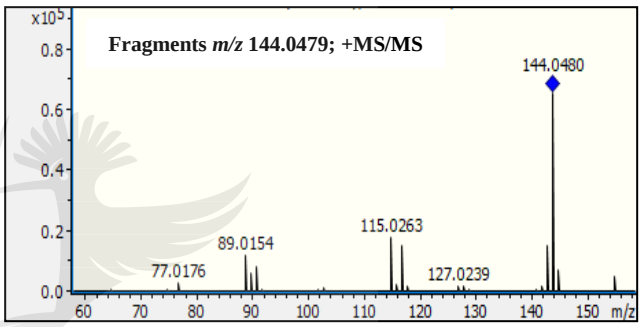
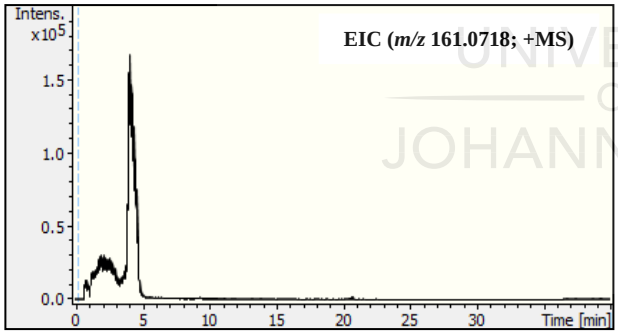
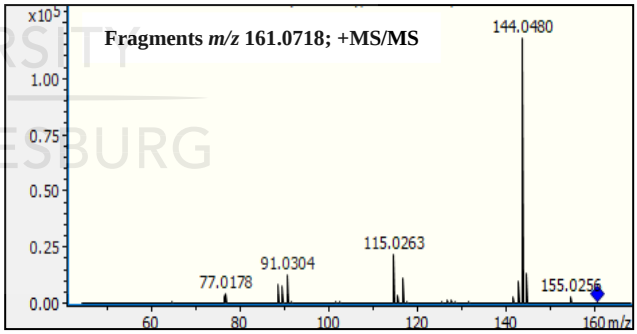
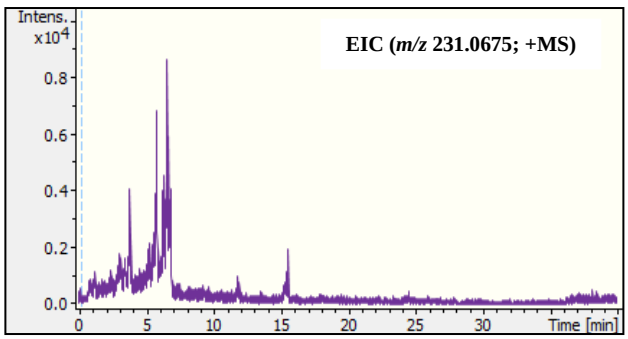
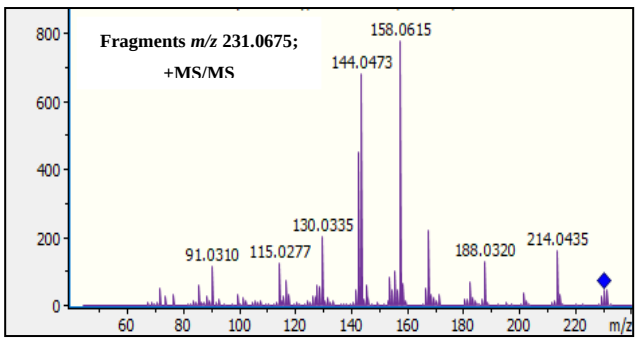
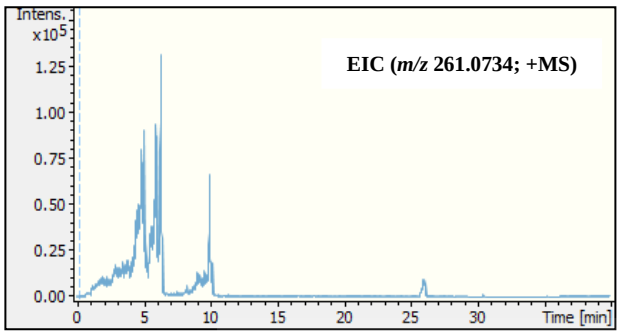
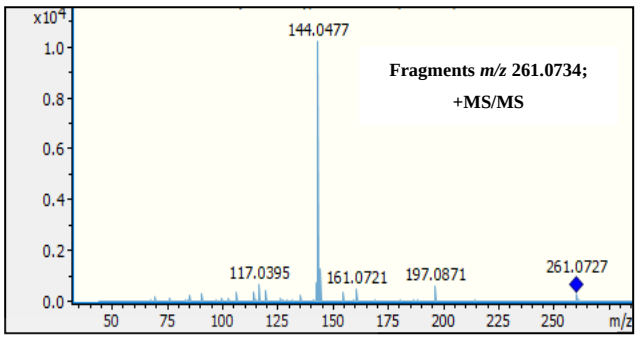
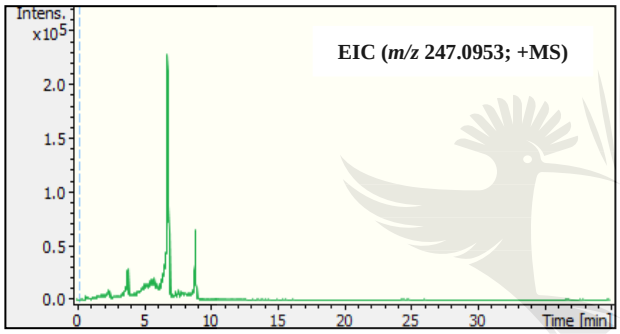
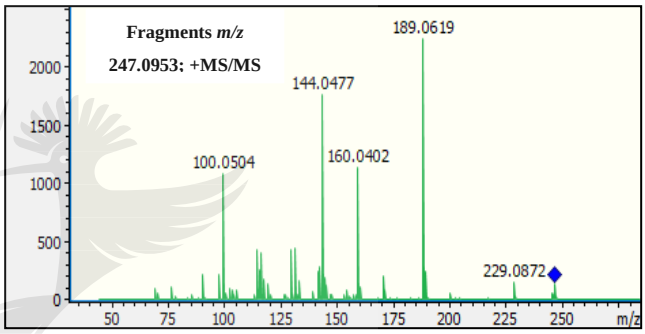
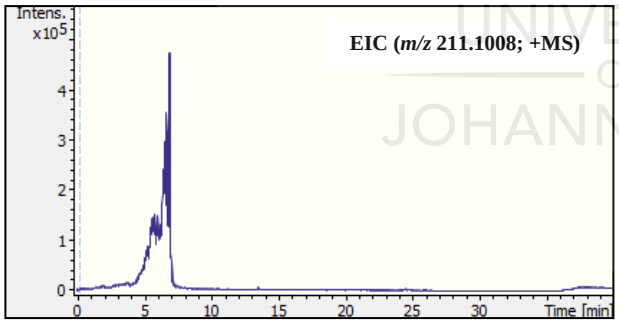
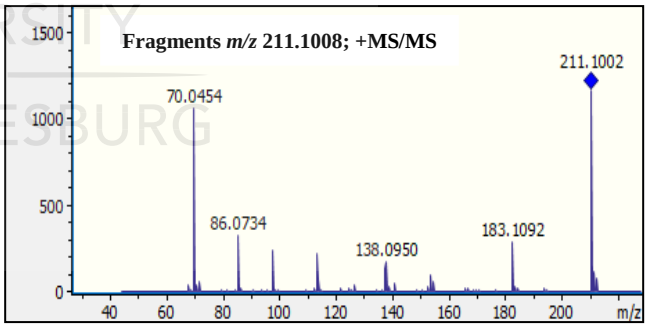
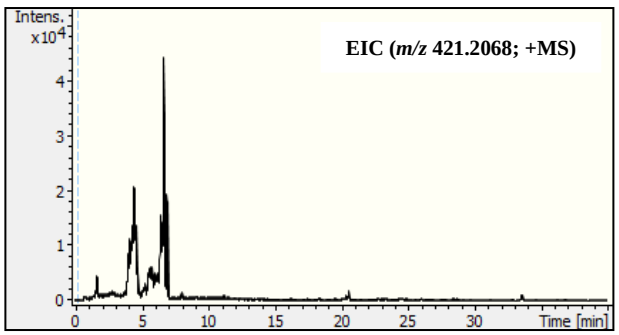
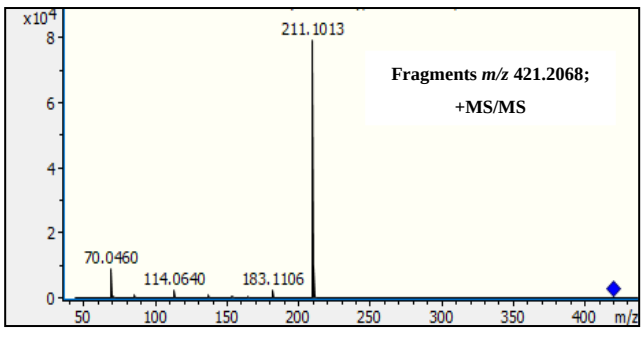
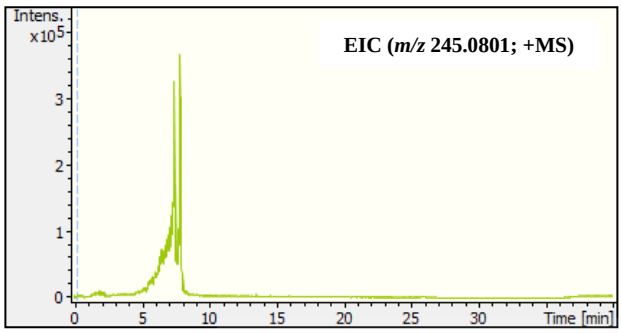
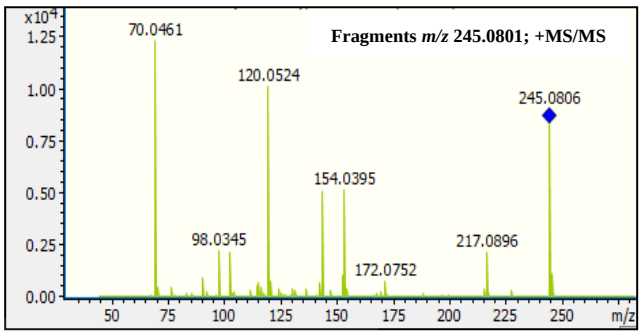
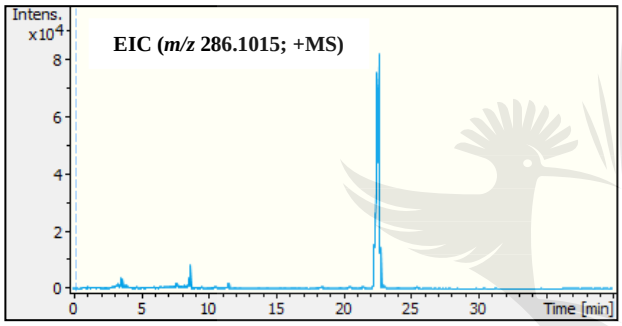
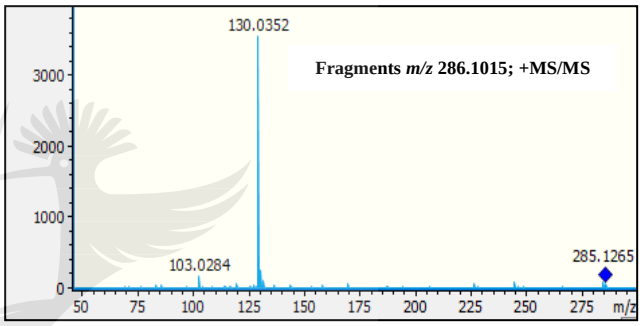
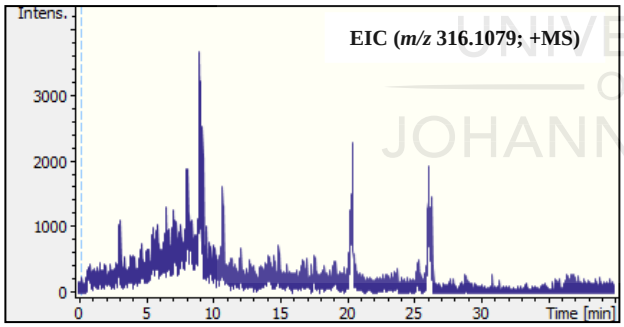
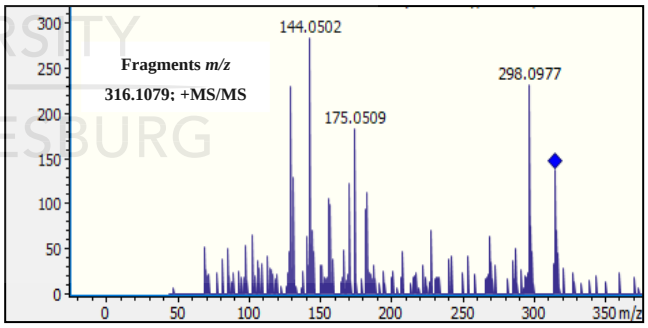
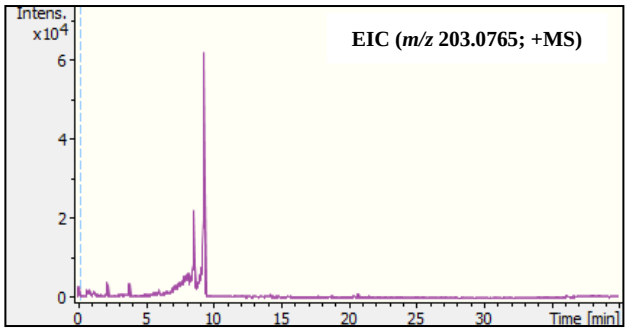
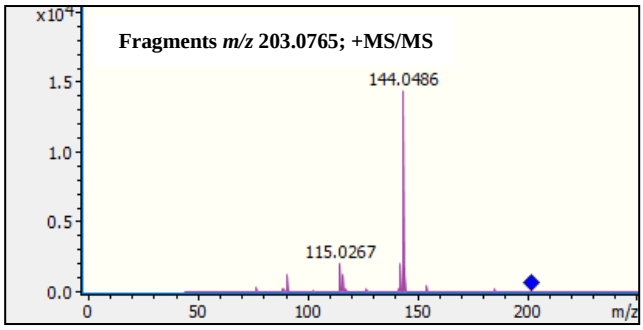
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Neoquassin 	

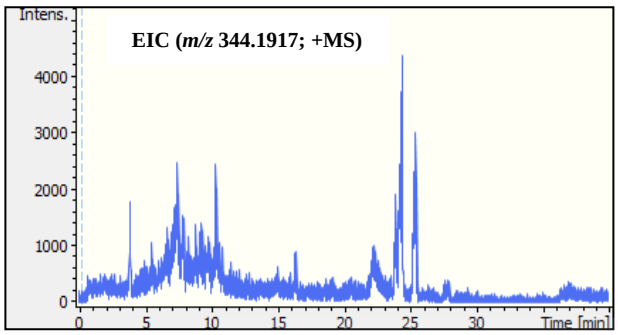
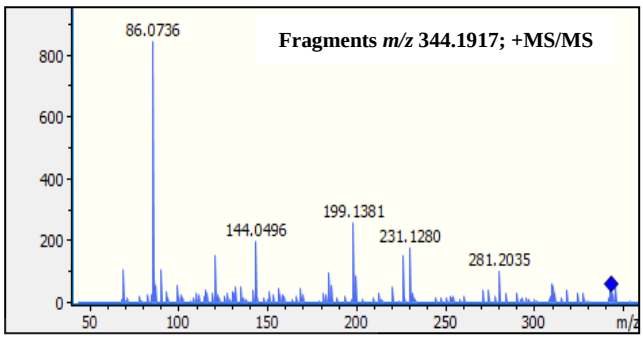
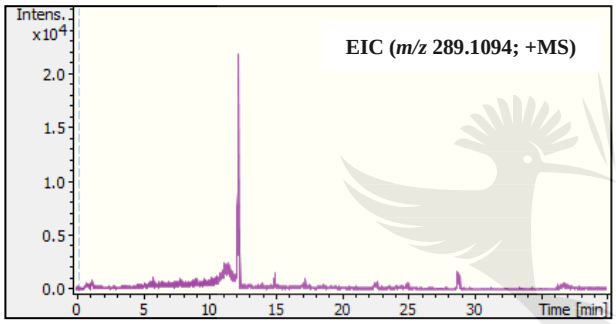
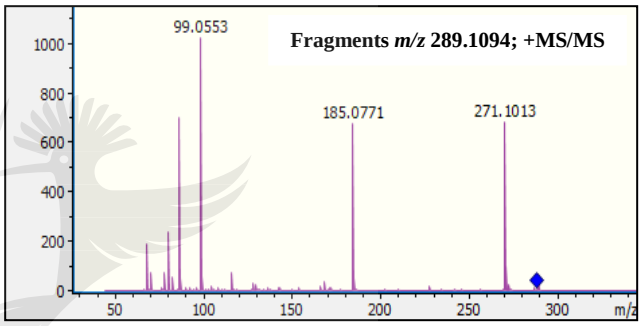
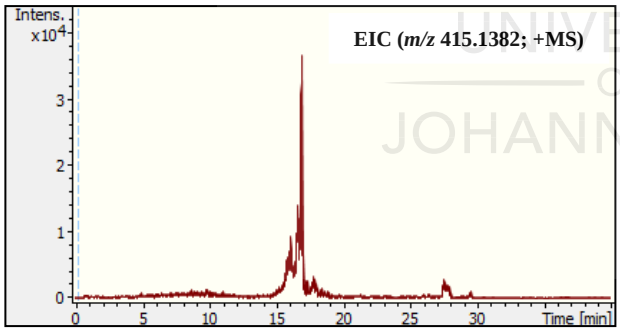
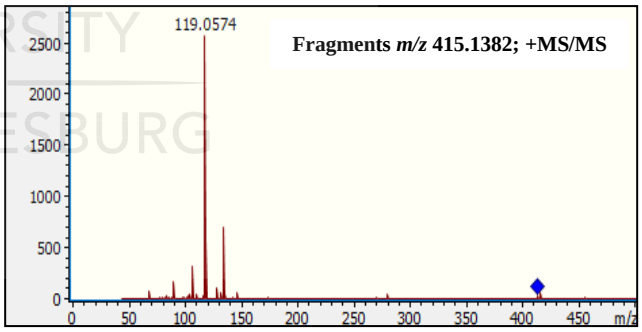
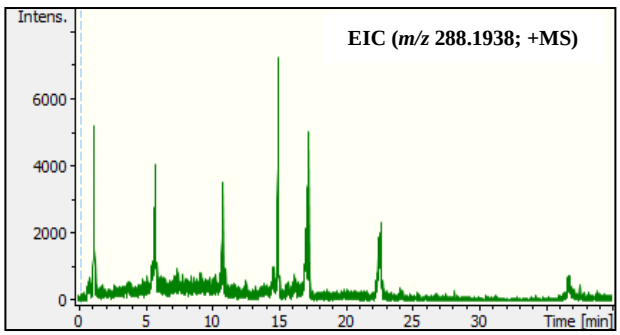
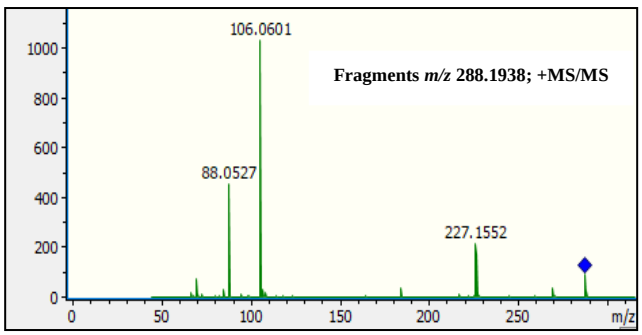
Table A6: Extracted ion chromatogram (EIC) of secondary metabolites from *Bacillus licheniformis* – NU 06 (left) and their MS^2 fragments (right).

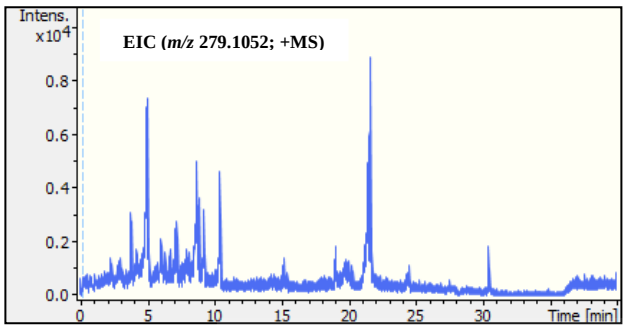
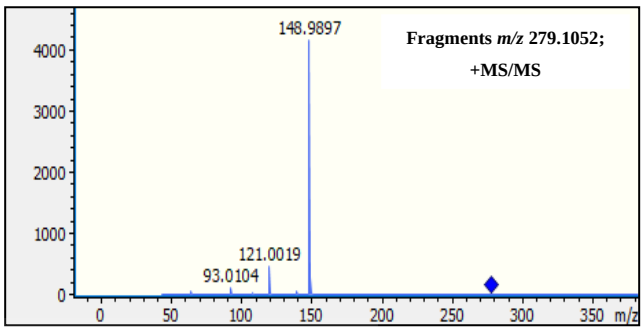
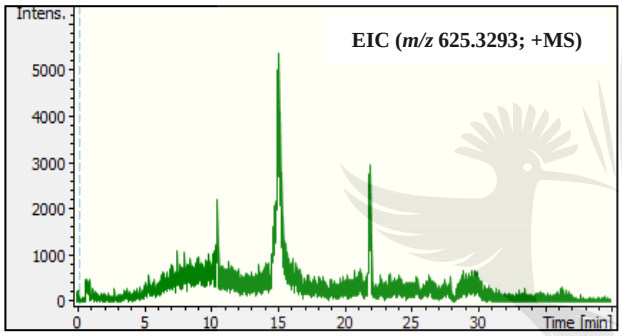
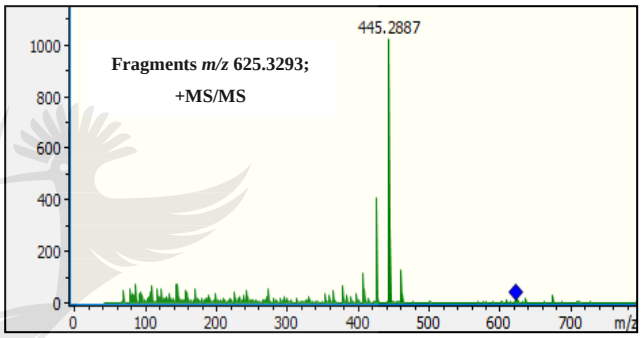
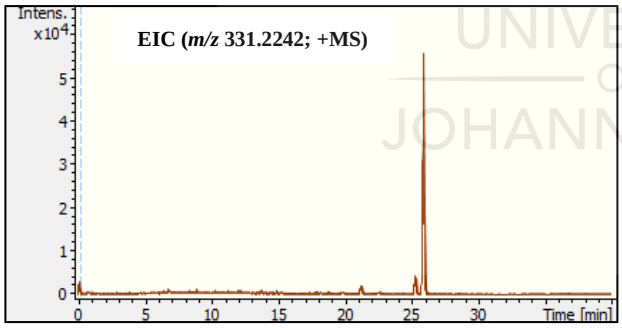
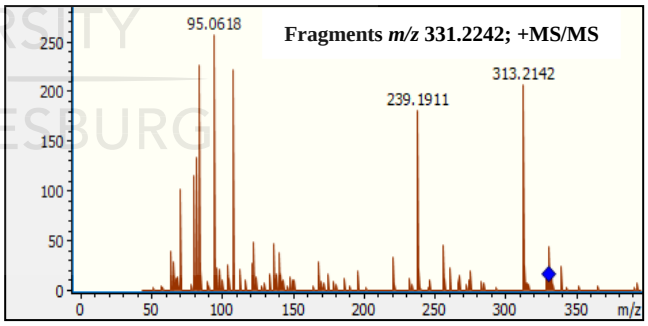
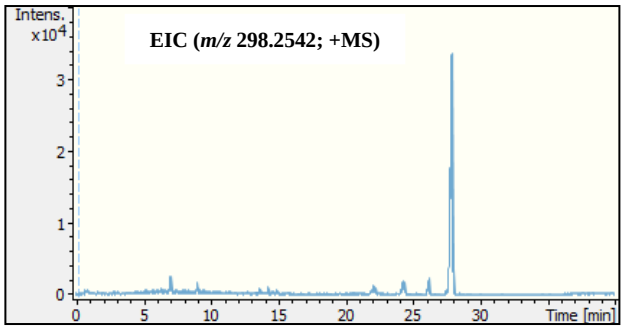
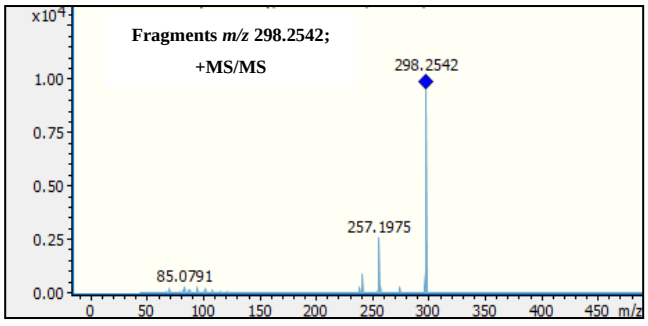
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Dubinidine	
	
Genipin	
	
Xanthosine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Droserone	
	
4-Methyl-5-(2'-hydroxyethyl)-thiazole	
	
Anatabine	
	
Visnagin	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Khellin	
	
(R)-Columbianetin	
	
Sinapyl alcohol	
	
Eupaformosanin	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Fulvoplumierin	
	
Arborinine	
	
Rosinidin	
	
Vasicinone	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
(-)-Laudanidine	
	
Asebogenin	
	
Picropodophyllin	
	
Isolobinine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Tuliposide A	
	
Dauricine	
	
Cannabielsoin	
	
Cassine	
	

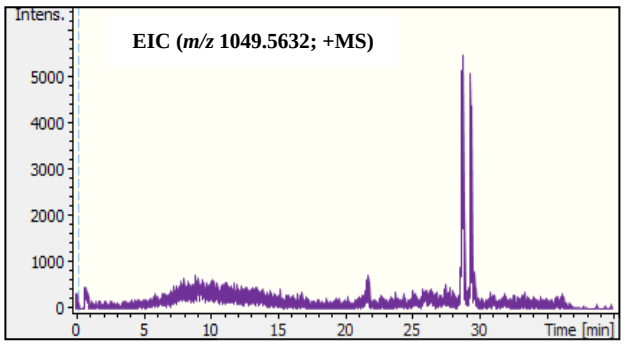
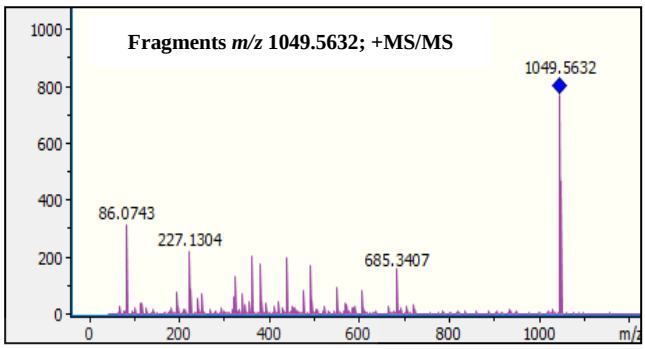
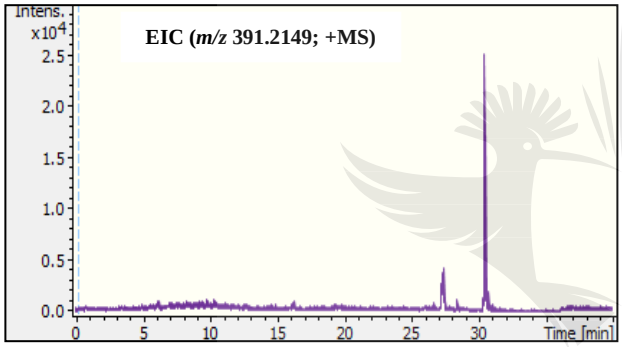
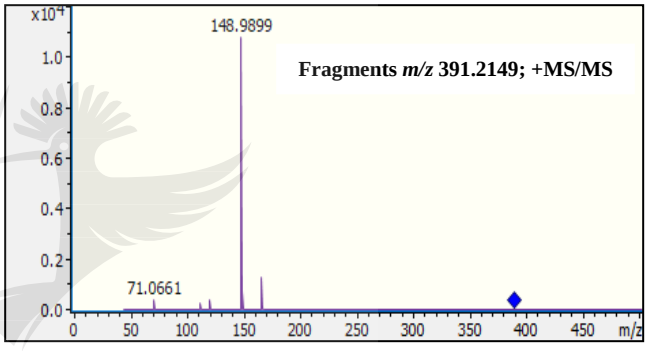
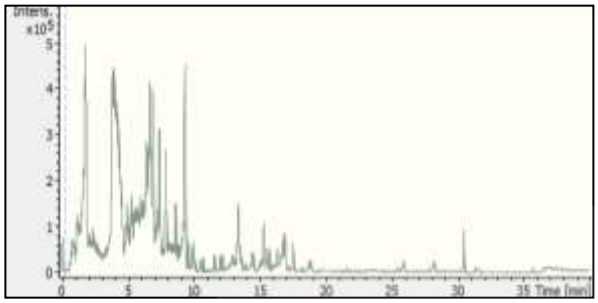
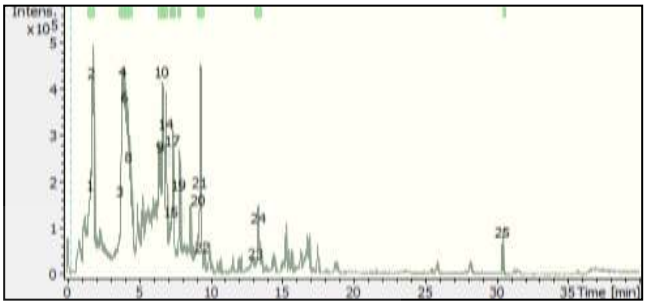
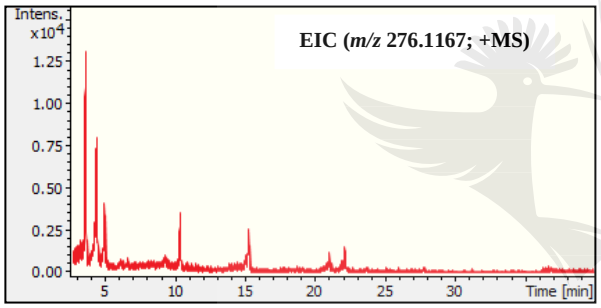
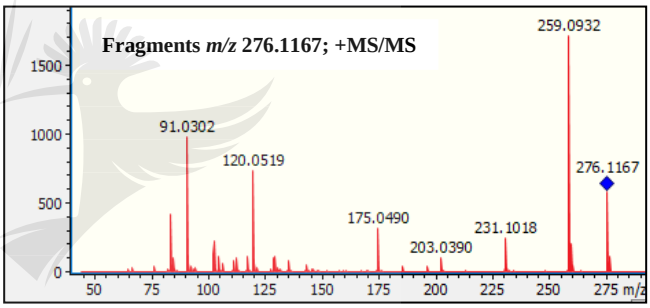
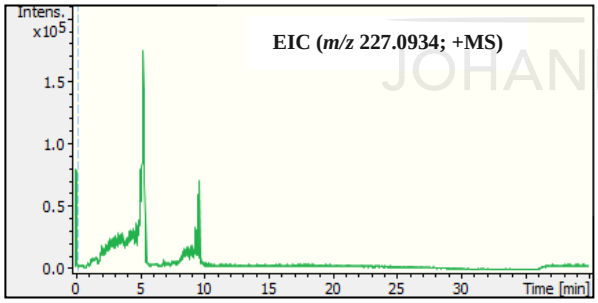
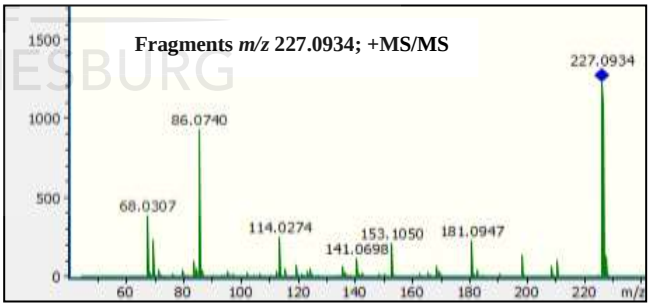
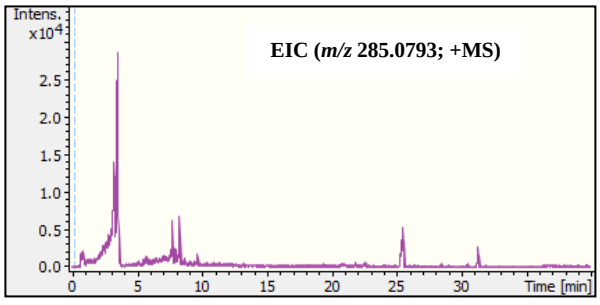
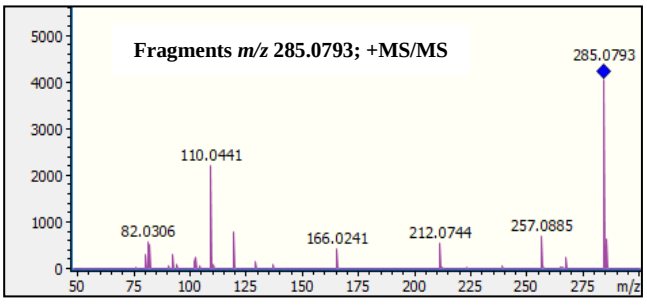
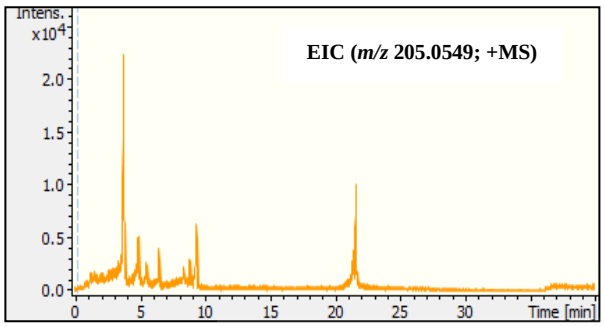
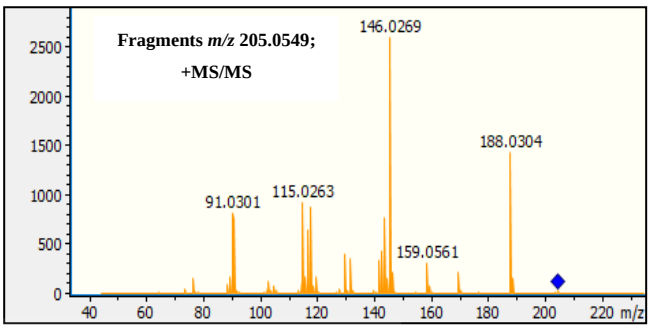
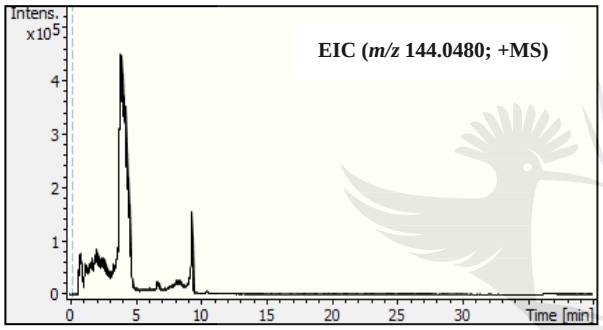
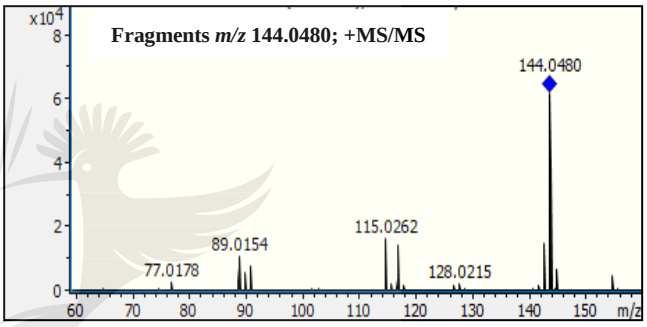
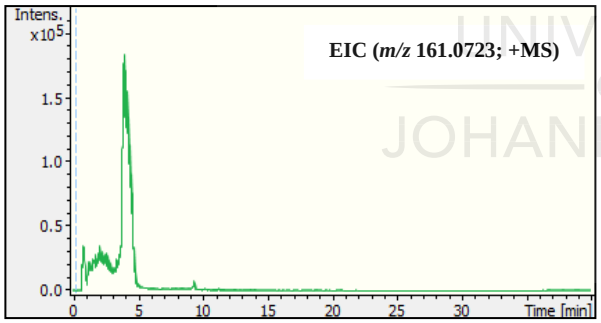
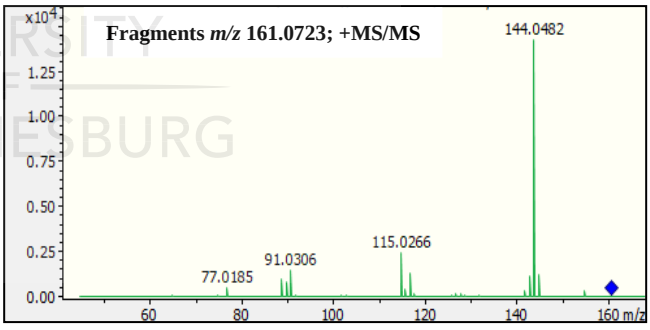
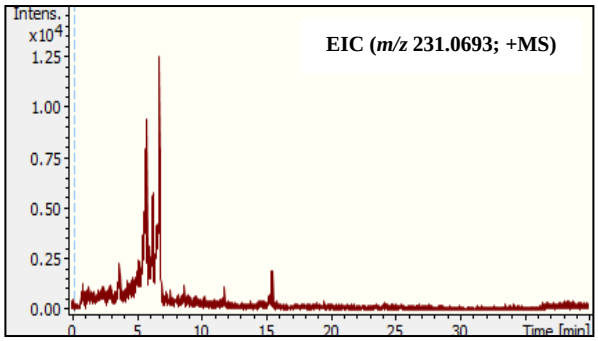
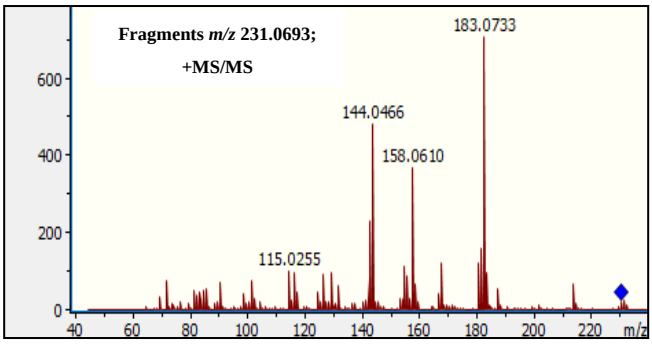
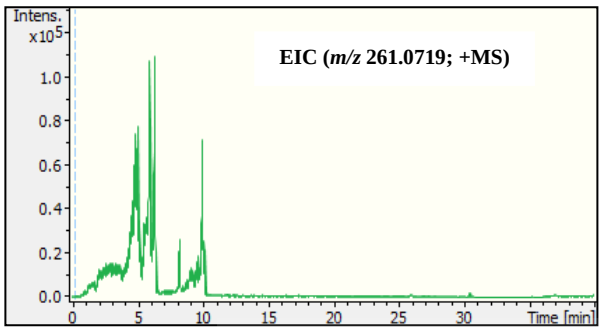
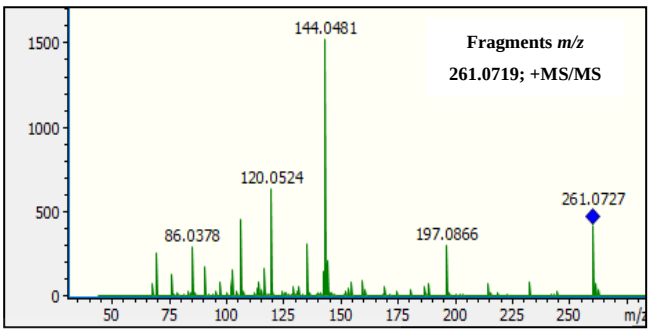
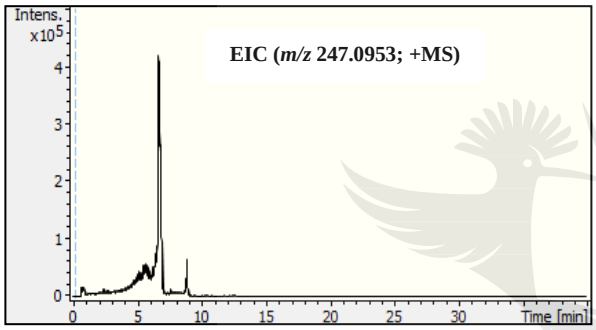
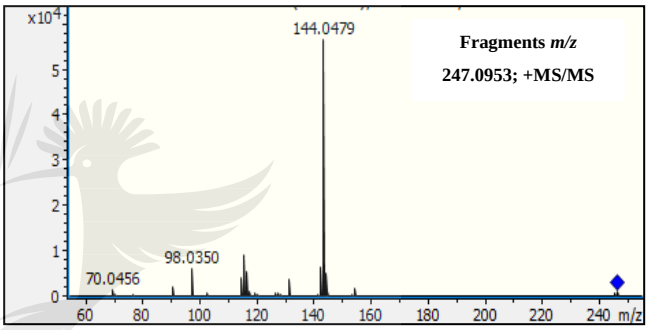
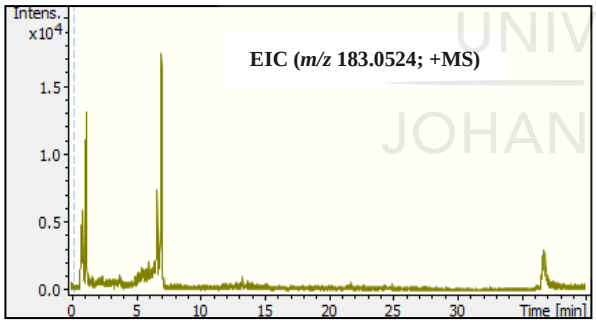
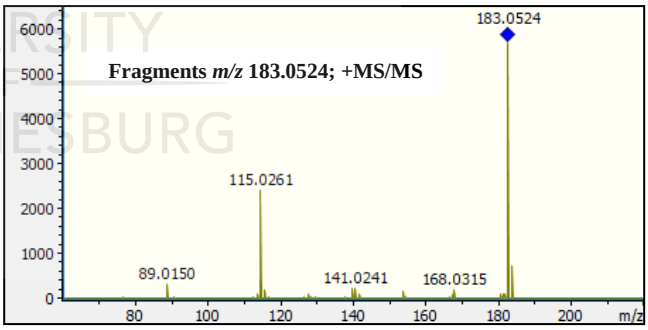
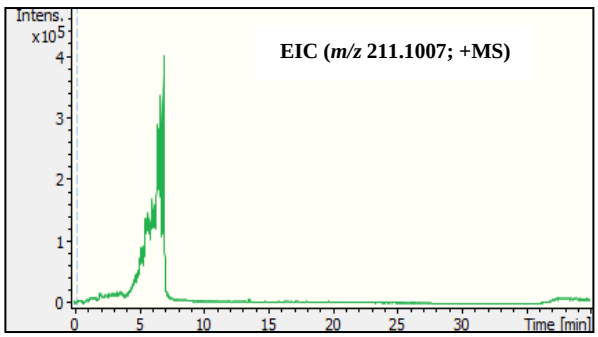
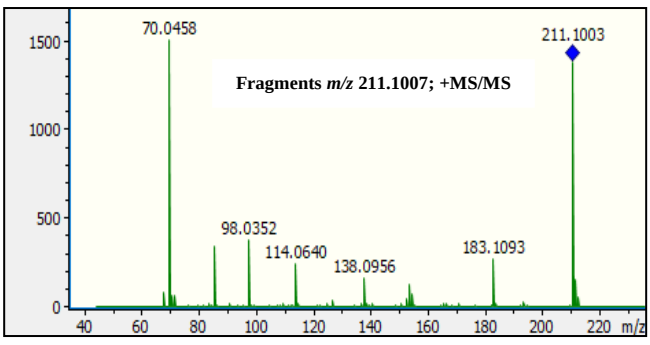
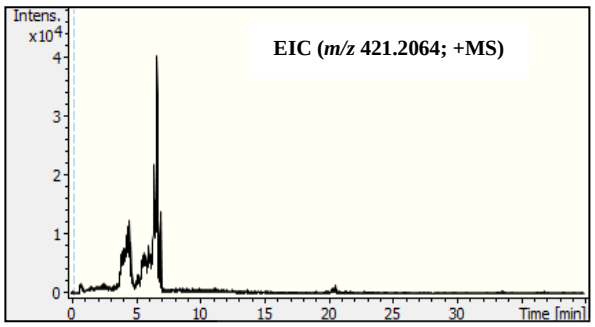
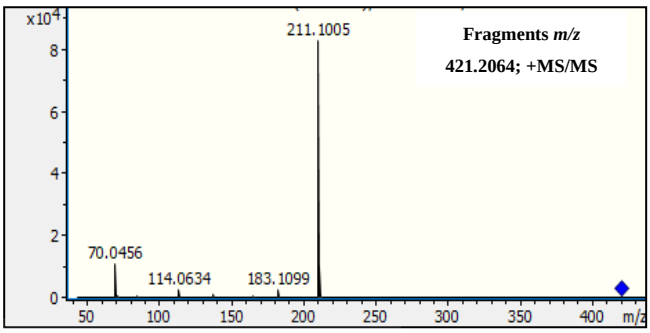
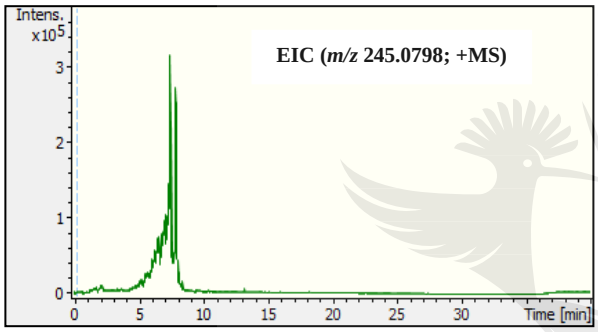
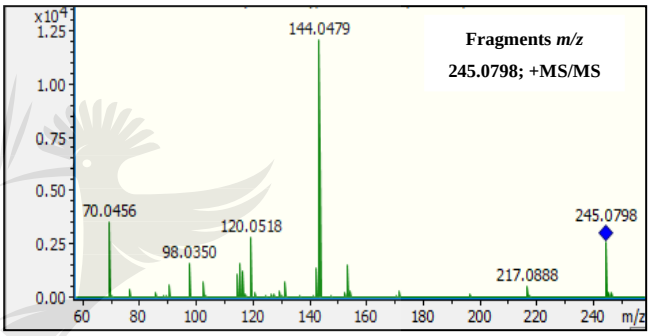
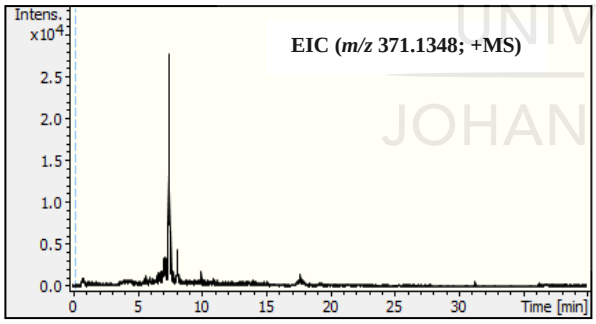
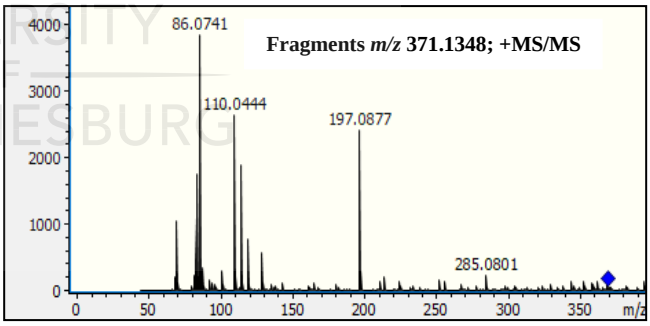
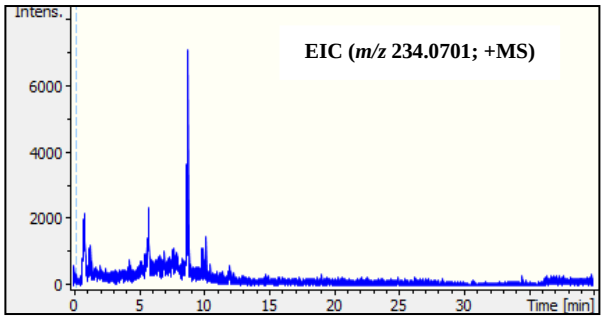
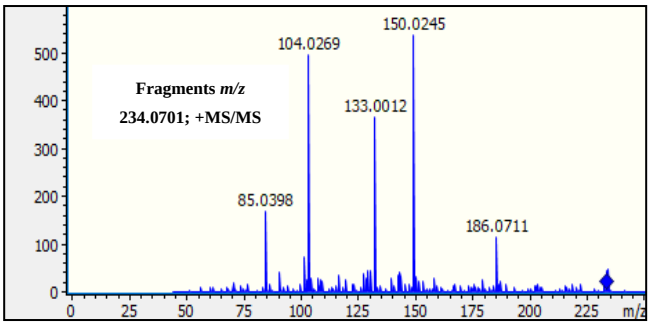
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Protodioscin	
	
Neoquassin	
	

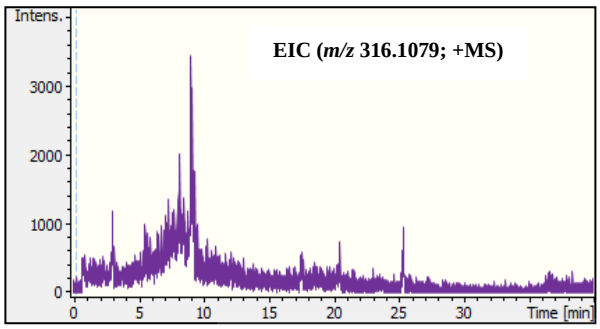
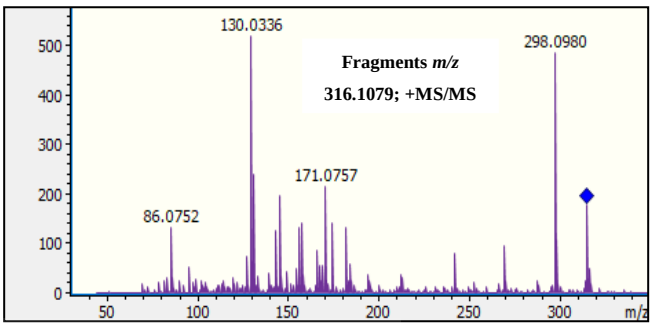
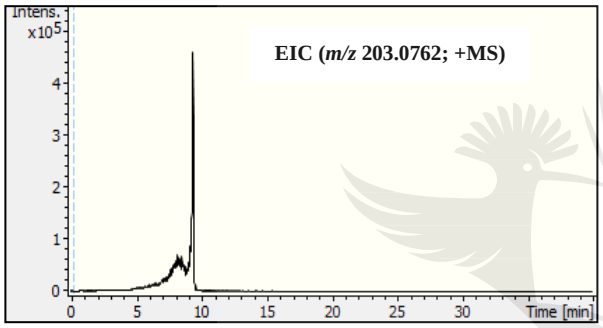
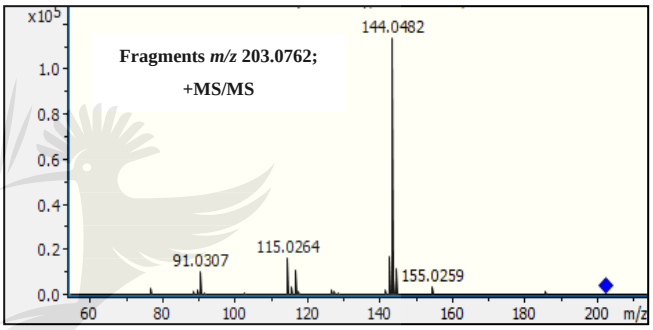
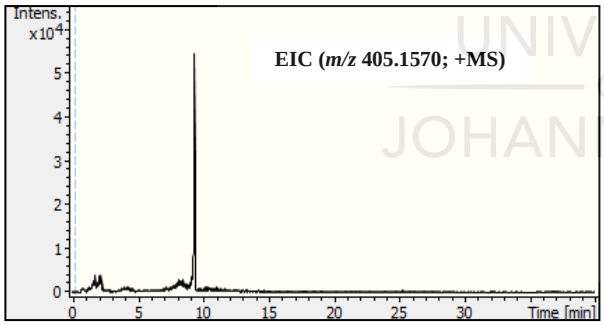
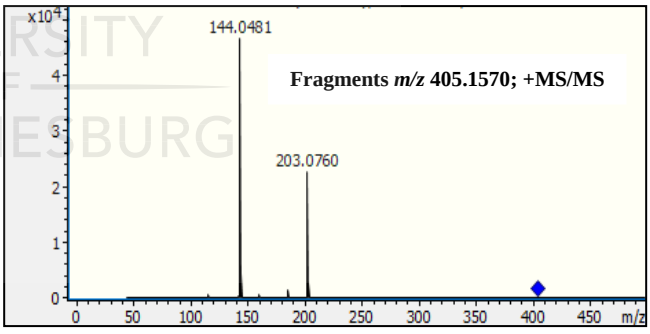
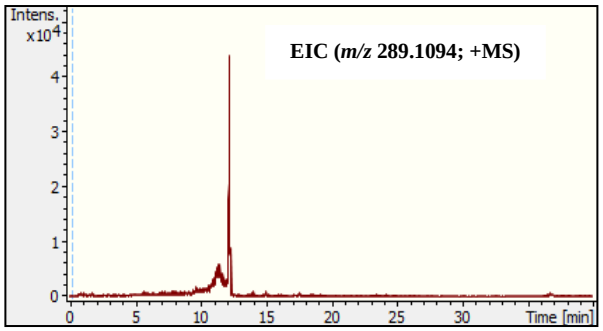
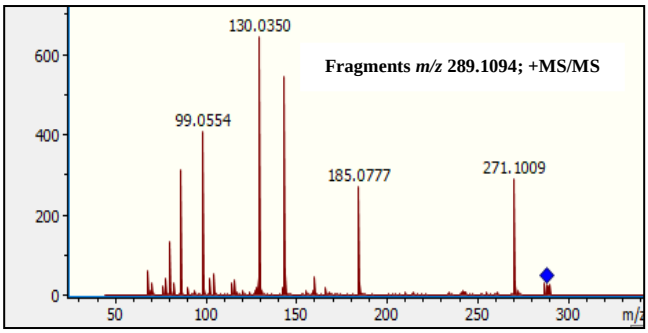
Table A7: Extracted ion chromatogram (EIC) of secondary metabolites from *Arthrobacter sp.* – NU 07 (left) and their MS^2 fragments (right).

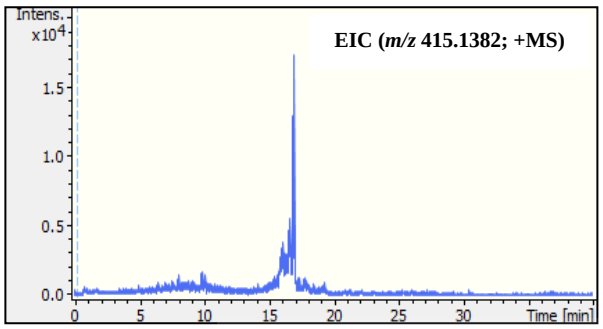
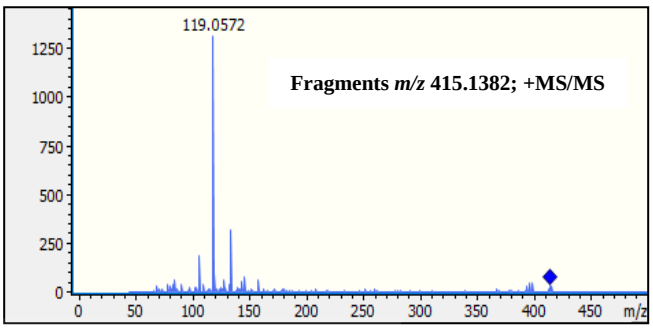
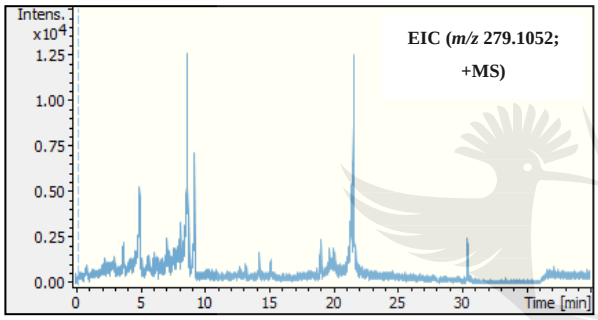
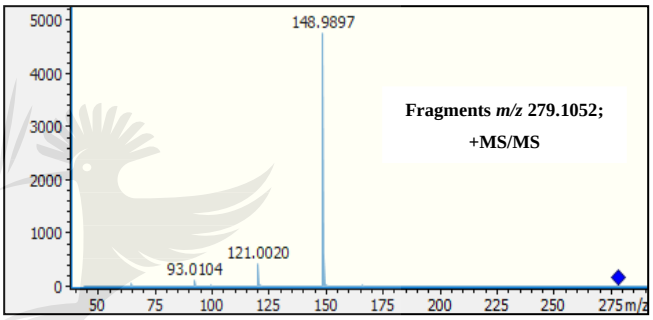
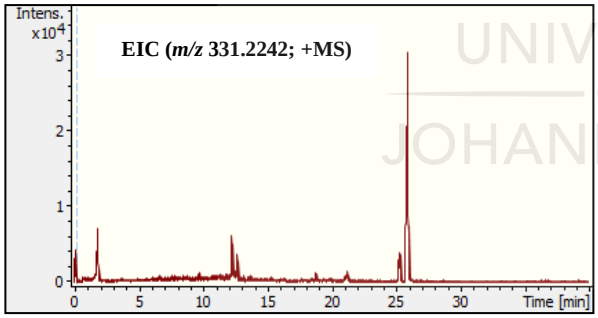
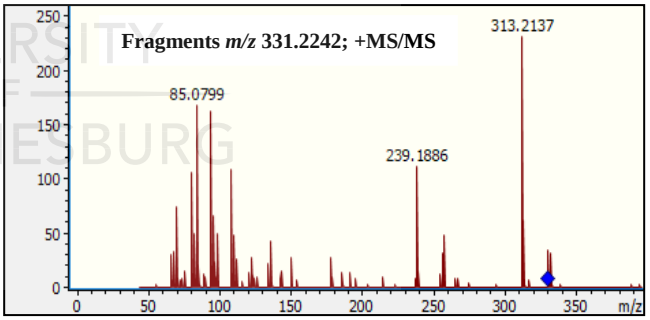
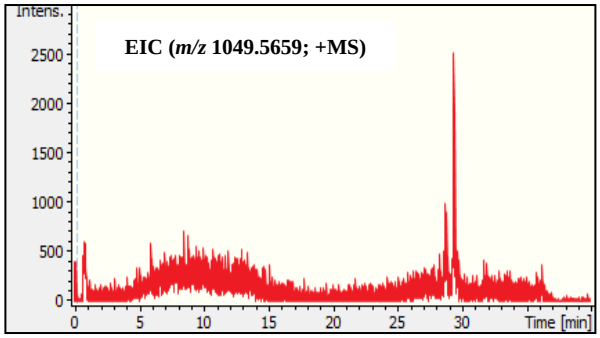
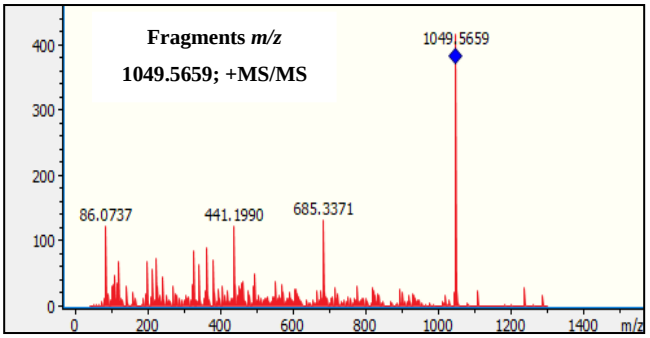
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Dubinidine	
	
Genipin	
	
Xanthosine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Droserone	
	
4-Methyl-5-(2'-hydroxyethyl)-thiazole	
	
Anatabine	
	
Visnagin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Khellin	
	
(R)-Columbianetin	
	
1-Methyluric acid	
	
Sinapyl alcohol	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Eupaformosanin	
	
Fulvoplumierin	
	
Sophoraisoflavanone A	
	
Casimiroin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Rosinidin	
	
Vasicinone	
	
Mallotophenone	
	
Asebogenin	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Picropodophyllin	
	
Tuliposide A	
	
Cannabielsoin	
	
Protodioscin	
	

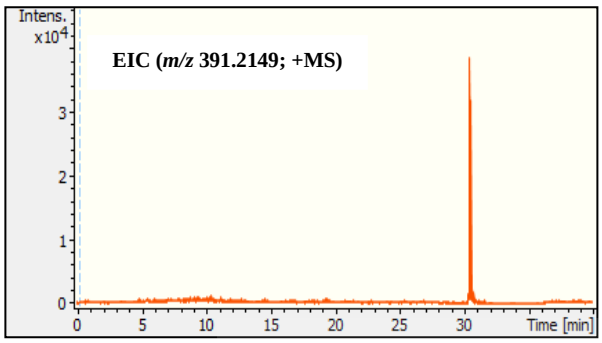
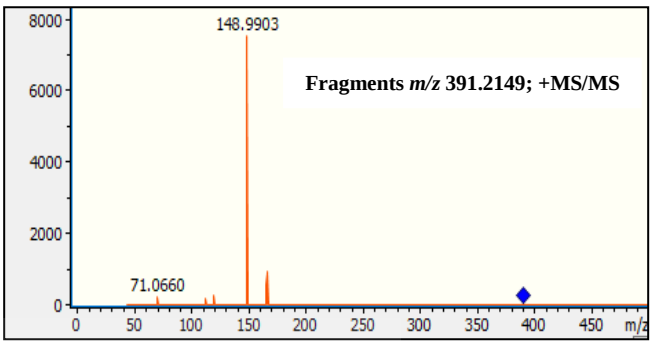
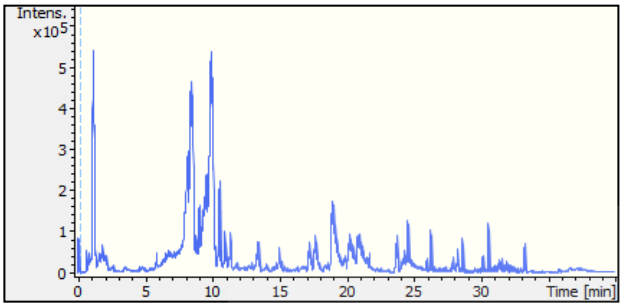
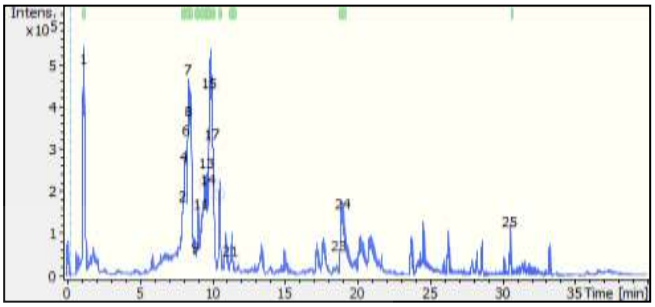
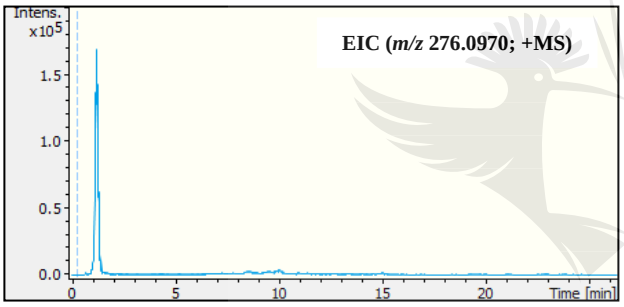
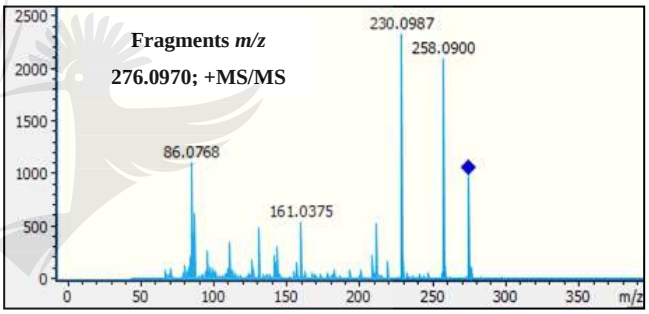
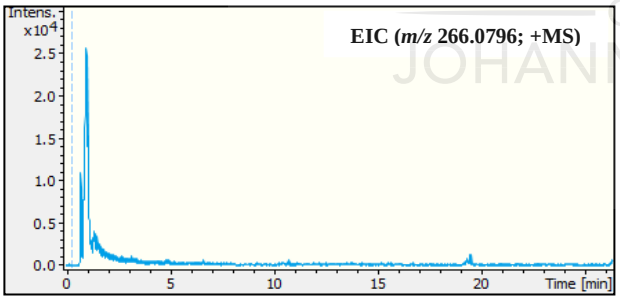
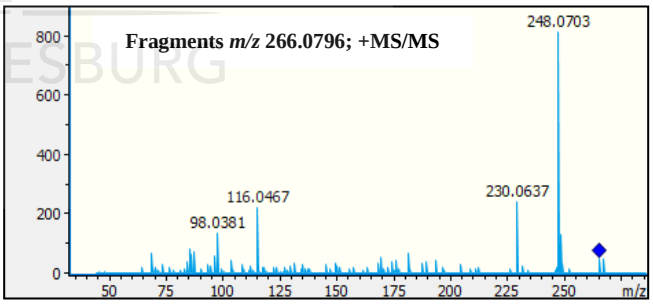
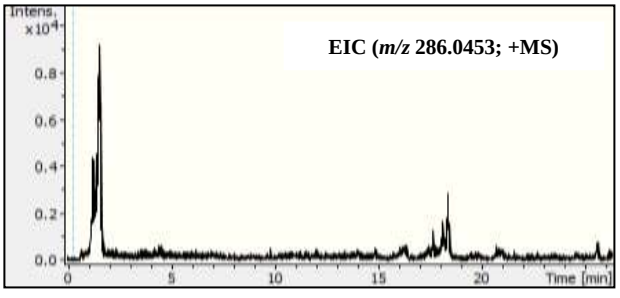
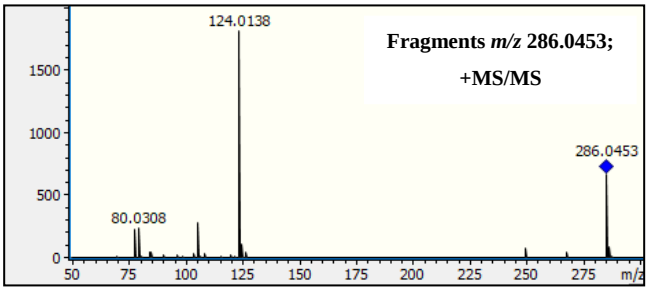
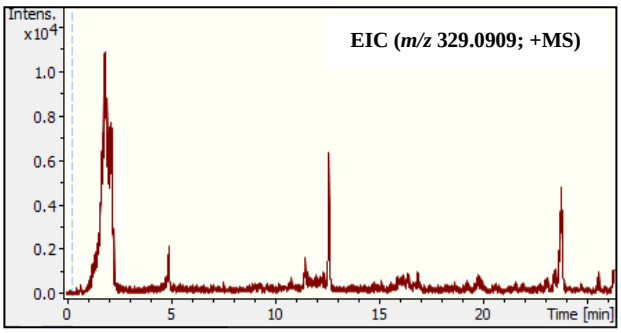
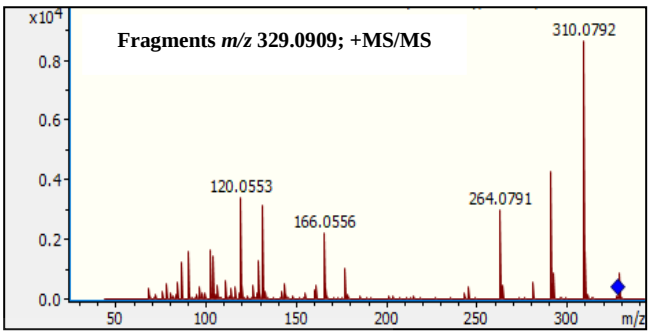
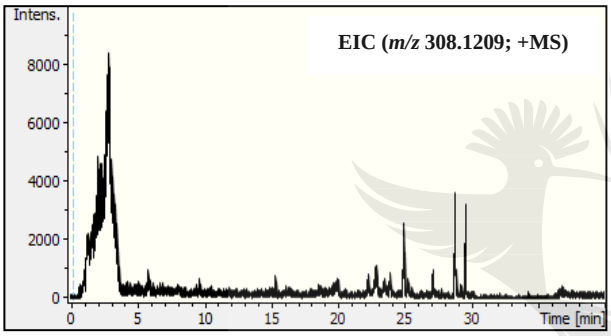
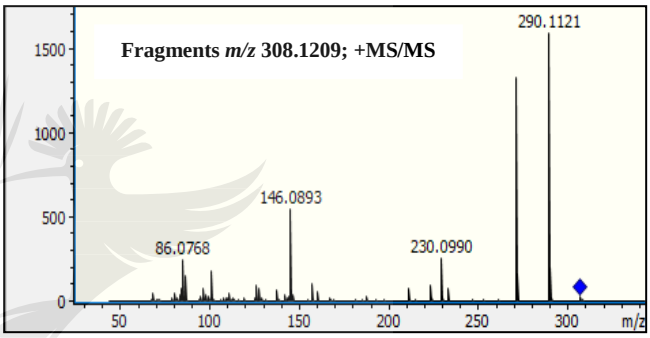
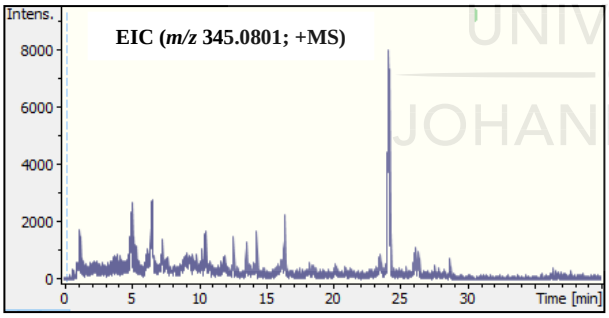
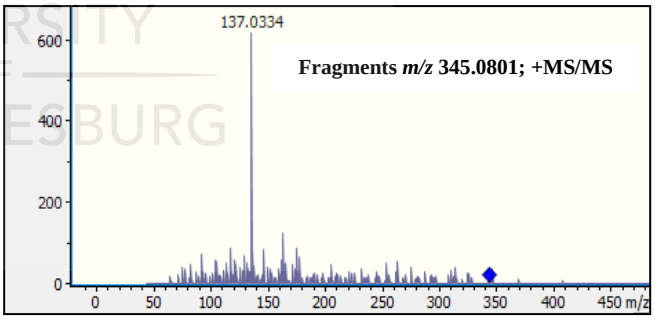
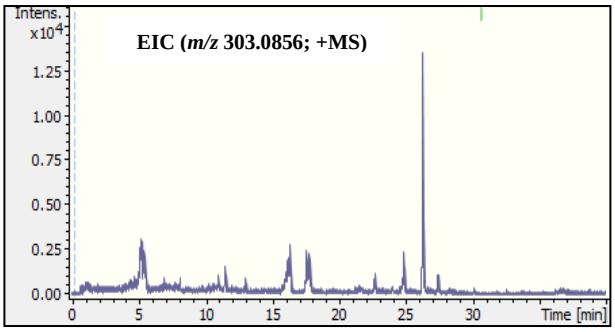
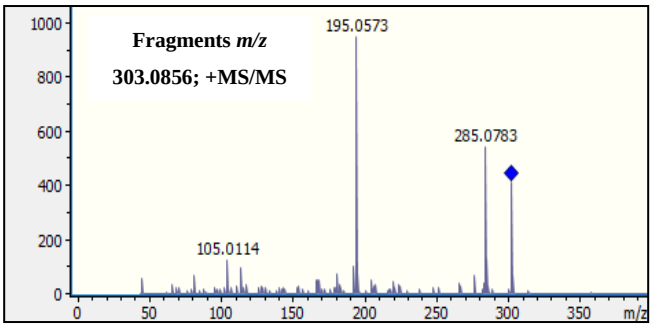
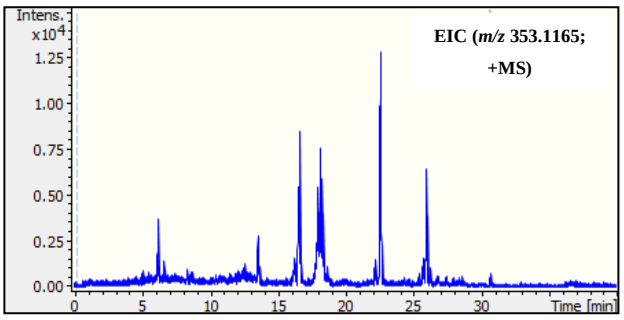
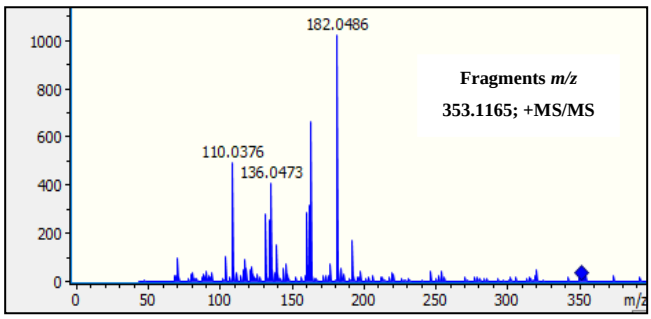
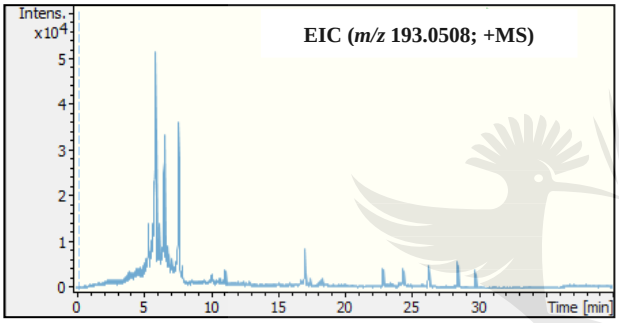
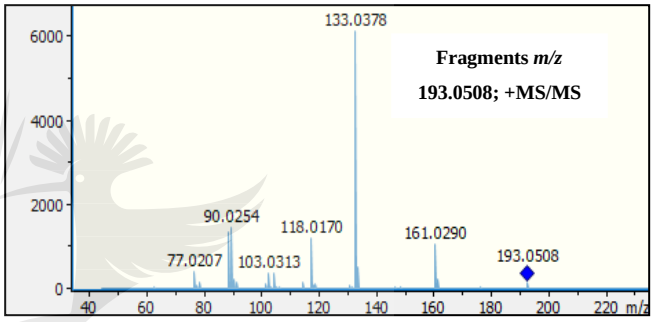
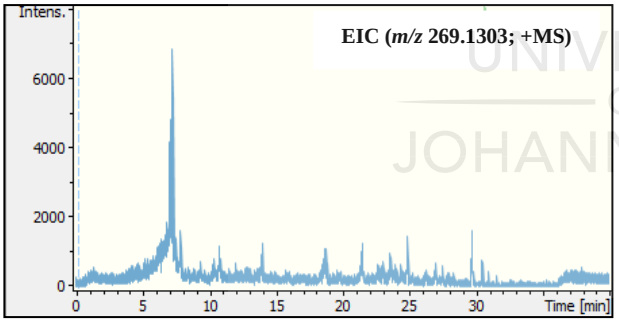
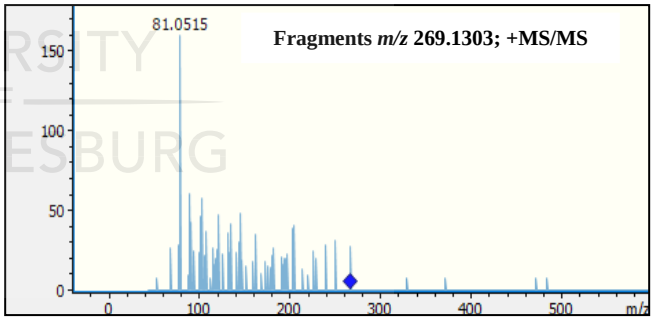
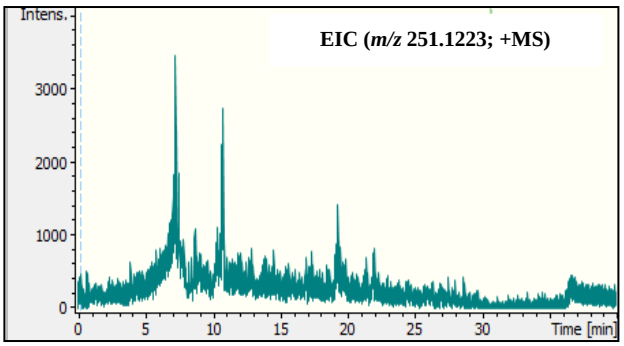
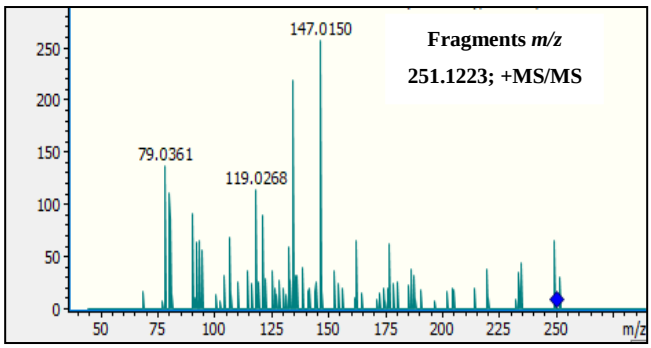
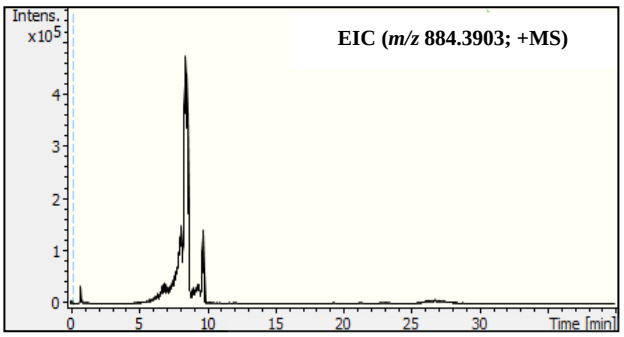
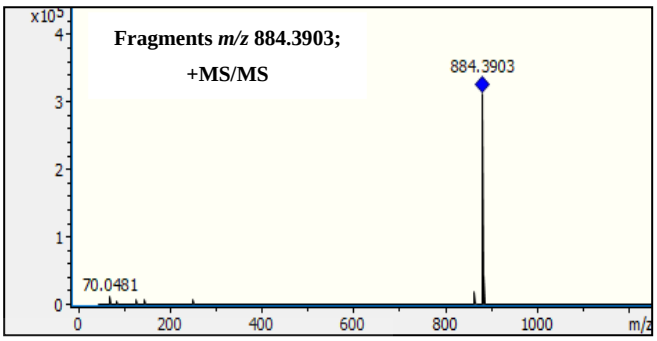
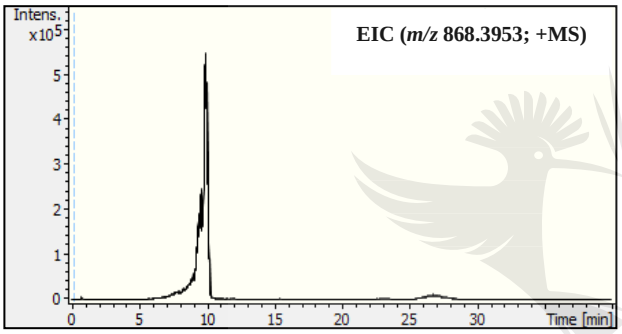
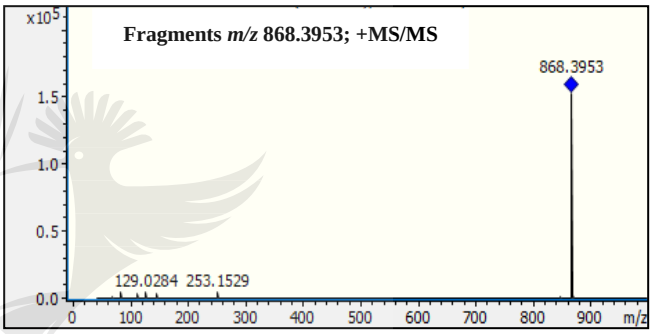
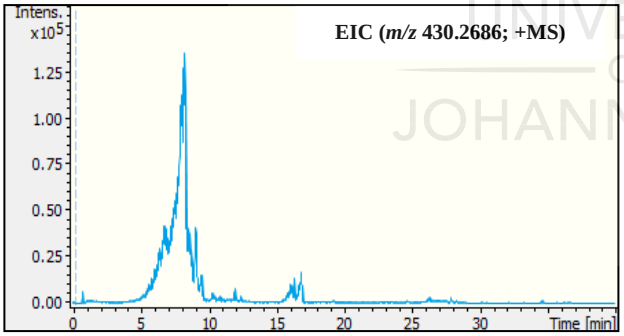
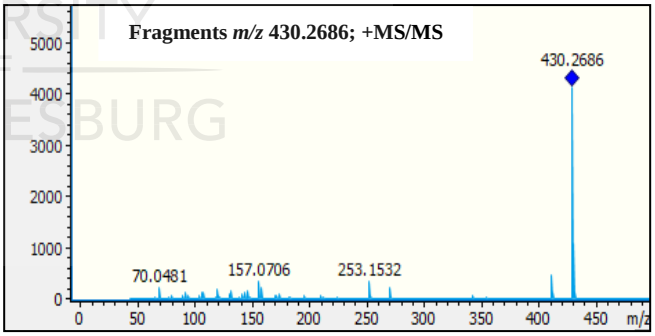
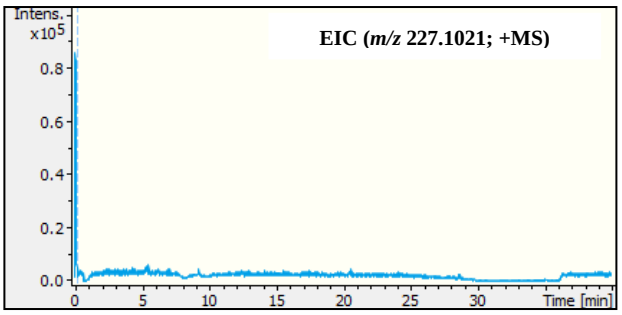
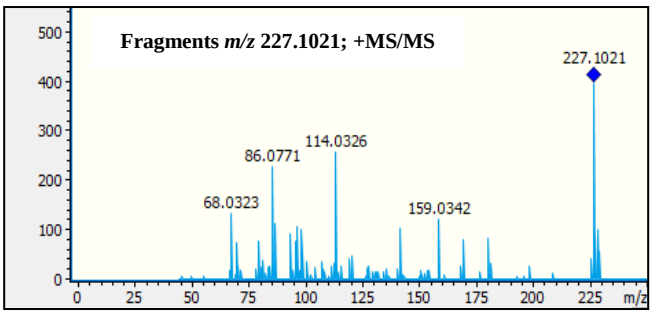
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Neoquassin 	

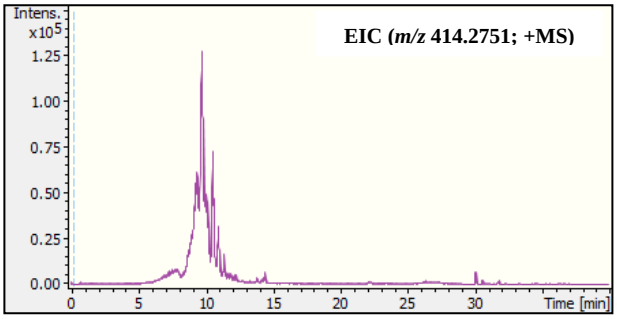
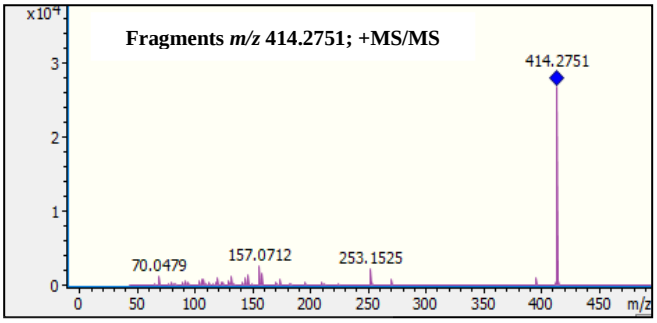
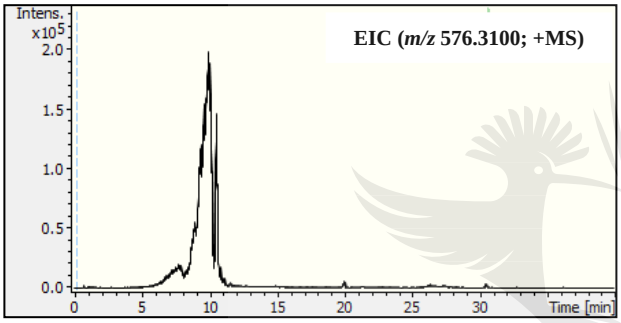
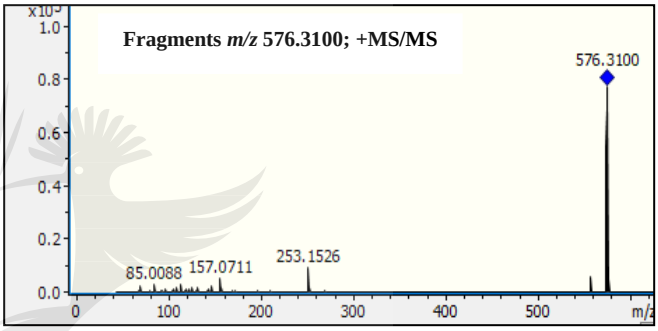
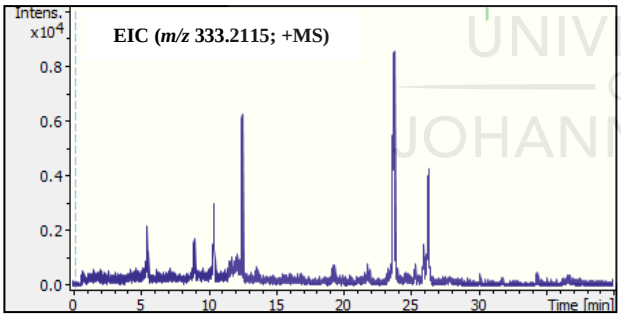
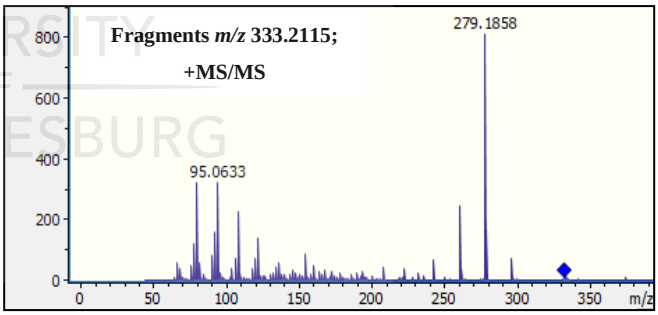
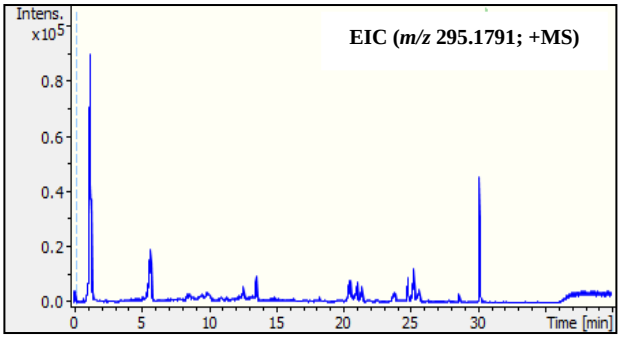
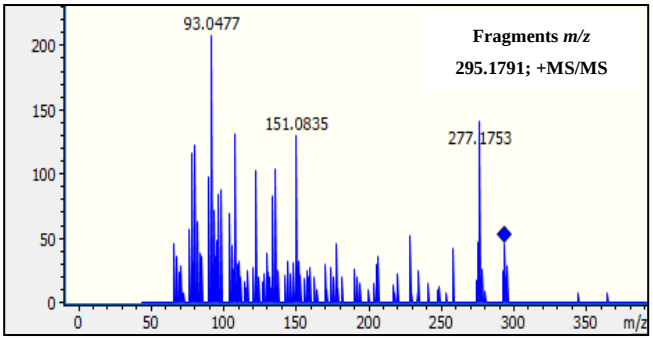
Table A8: Extracted ion chromatogram (EIC) of secondary metabolites from *Solanum mauritianum* ripe fruits coat – S1 RFC (left) and their MS^2 fragments (right).

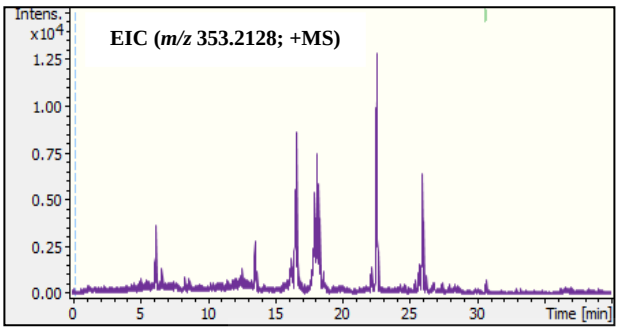
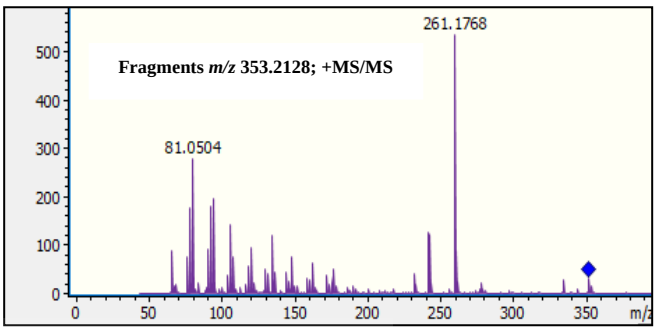
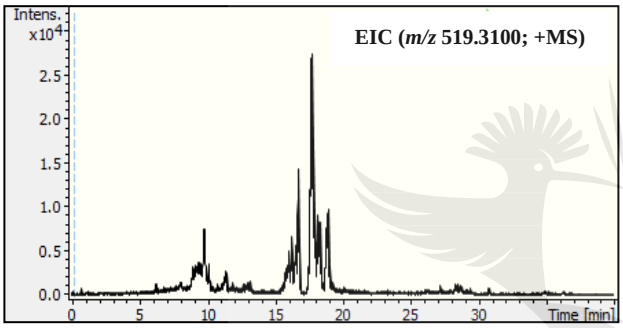
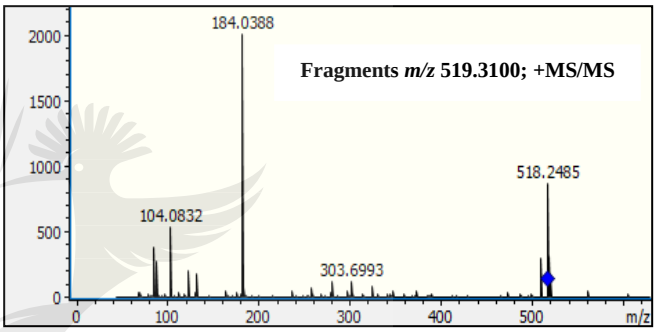
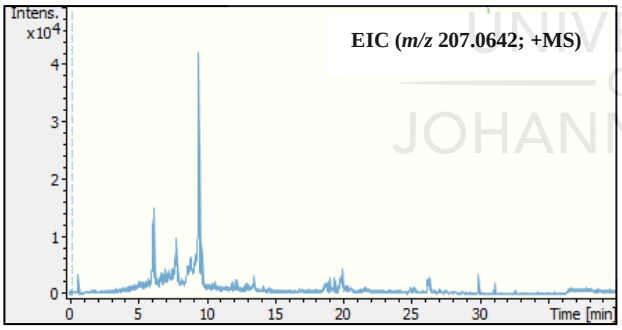
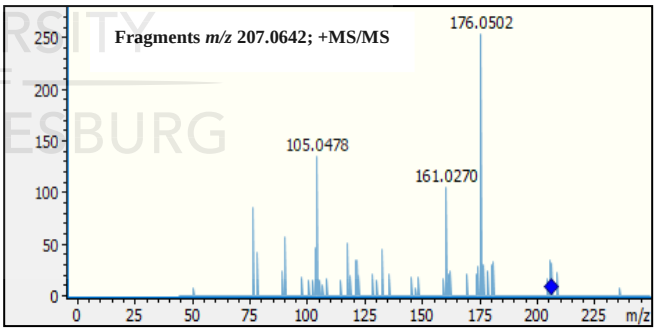
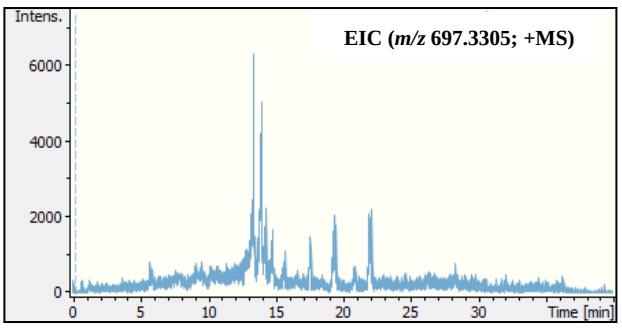
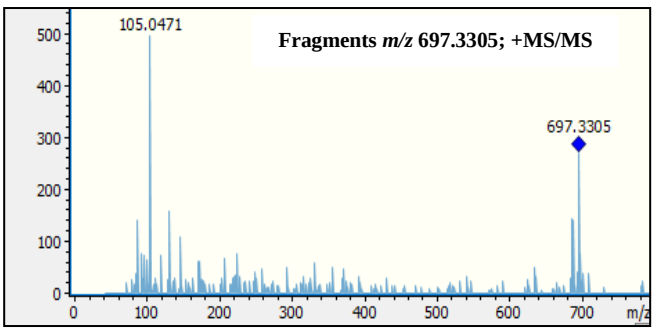
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram	
	
Cardiospermin	
	
Furofoline I	
	
Riccionidin A	
	

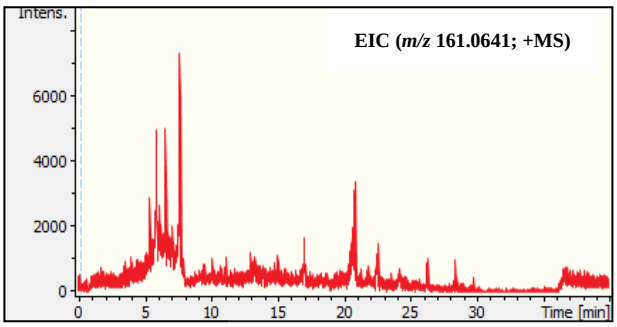
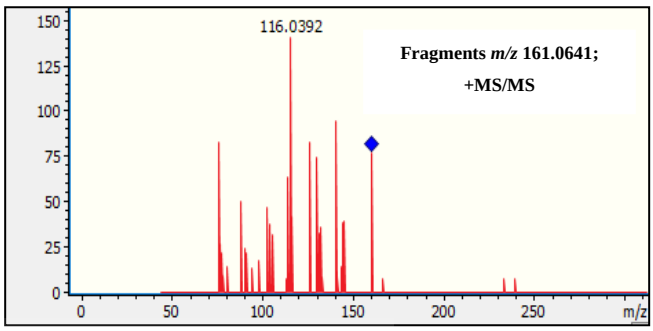
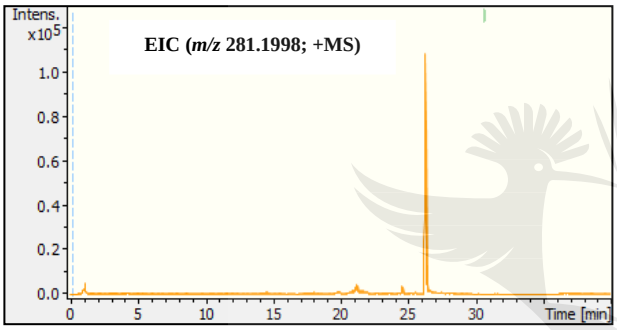
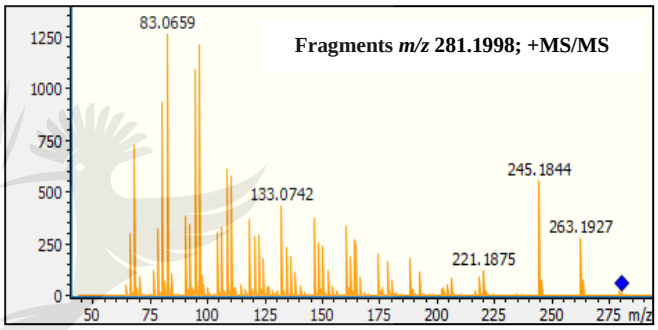
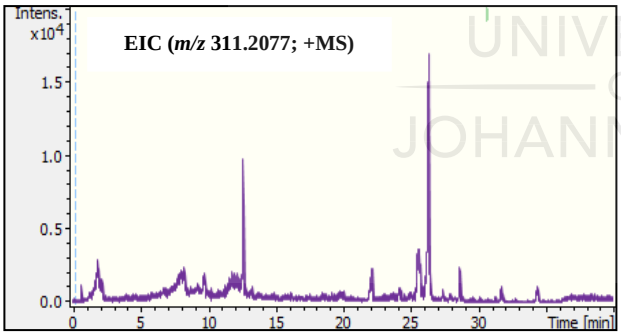
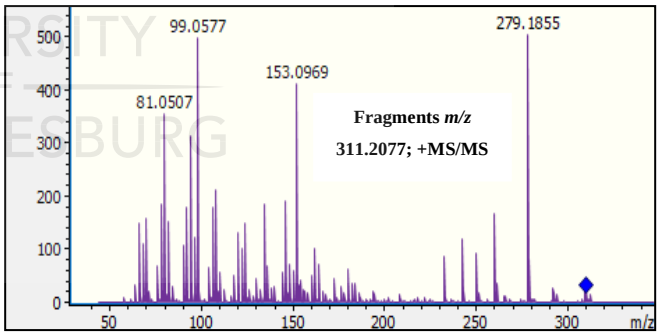
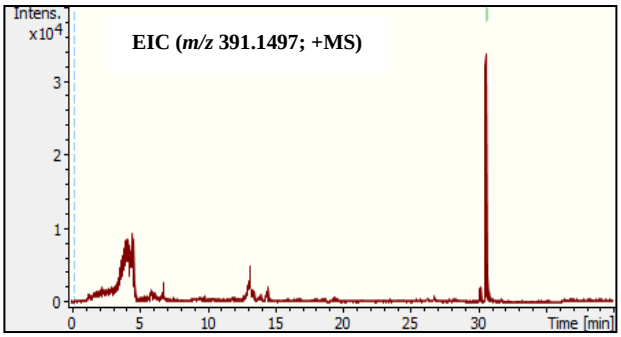
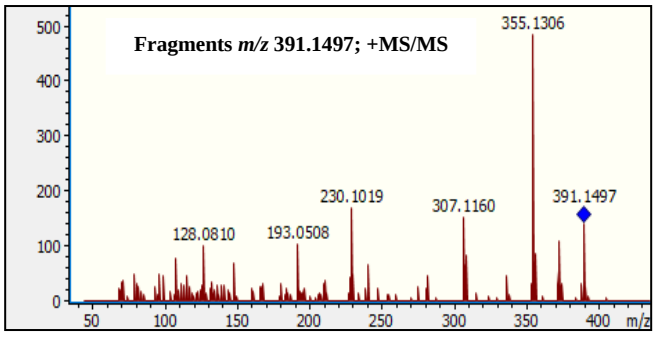
EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Bergenin	
	
Cusparine	
	
Theogallin	
	
Homoeriodictyol chalcone	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Berberastine	
	
Scopoletin	
	
Lysergic acid	
	
Glycophymoline	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Solasonine	
	
α-Solanine	
	
Imperialine	
	
Genipin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Solasodine	
	
α-Ergocryptine	
	
Callicarpone	
	
10-Deoxysarpagine	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Montanol	
	
Hypercalin B	
	
Eugenin	
	
Thalicarpine	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Anatabine	
	
Ibogamine	
	
Ibogaine	
	
Loganin	
	

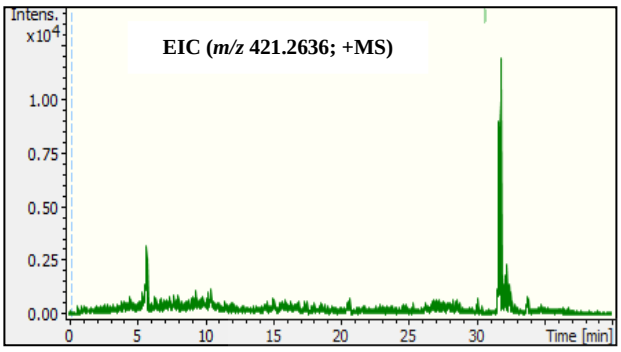
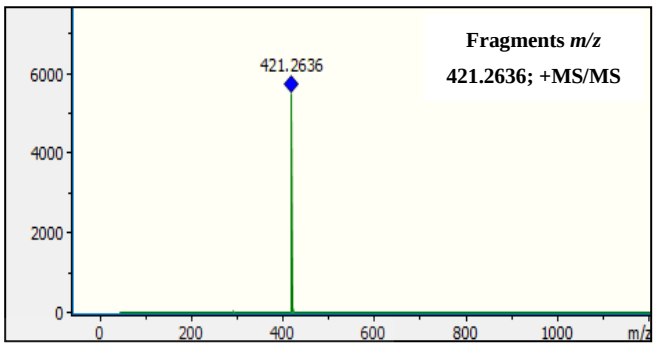
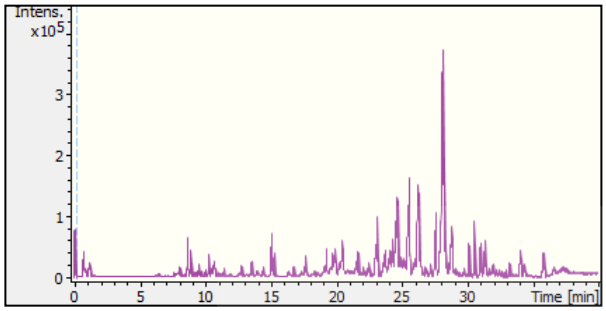
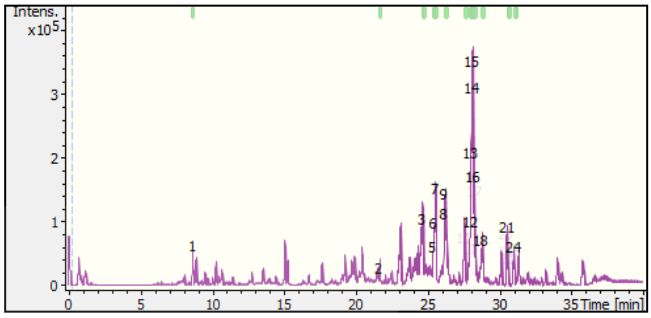
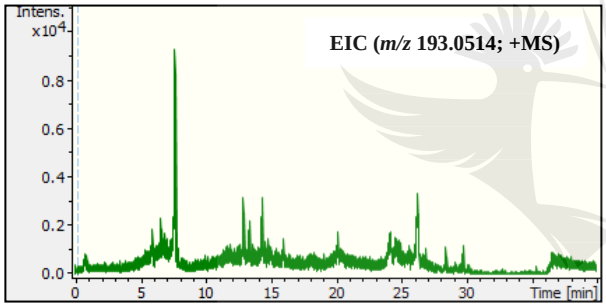
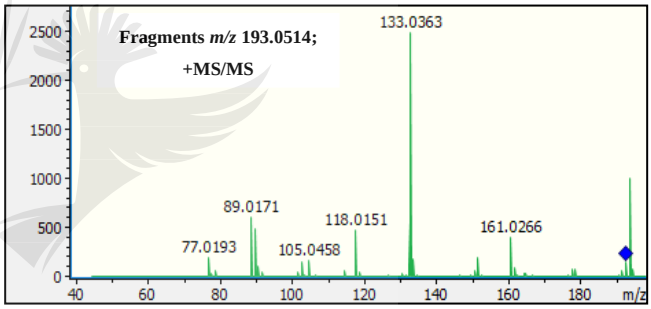
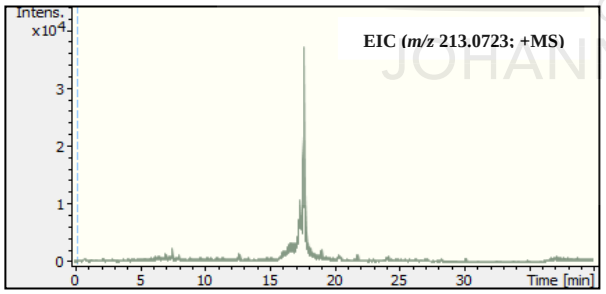
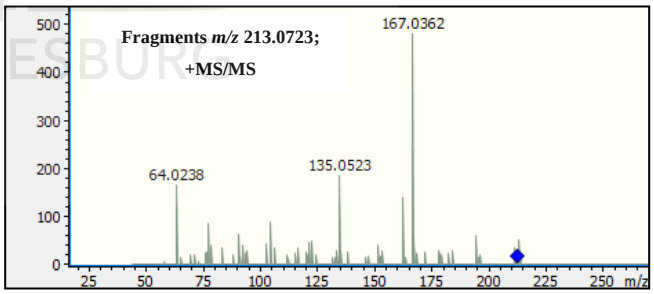
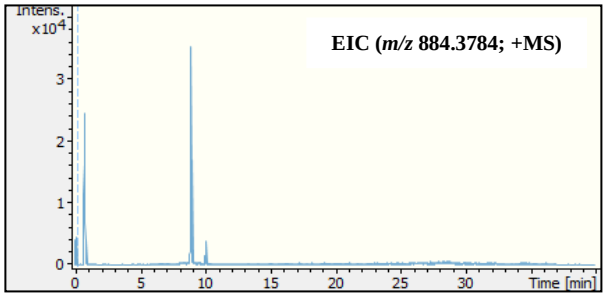
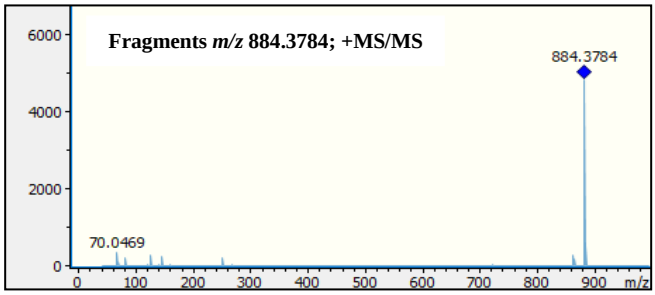
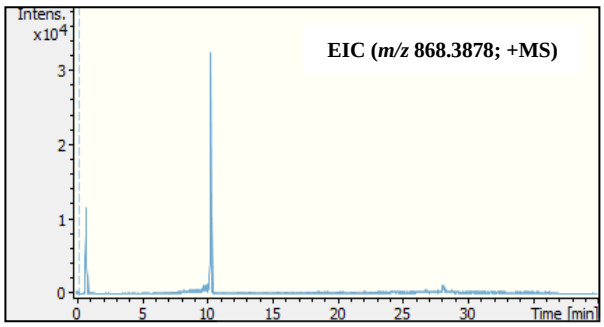
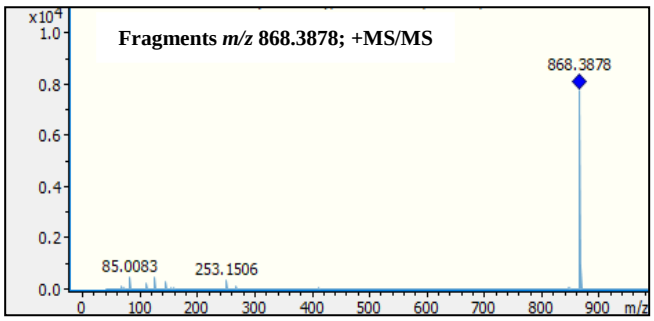
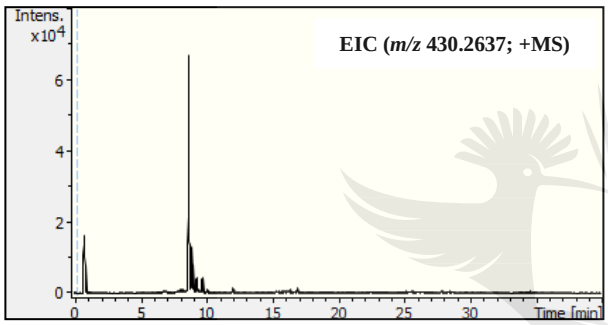
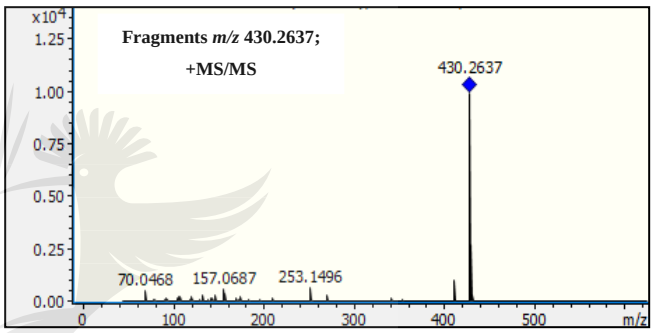
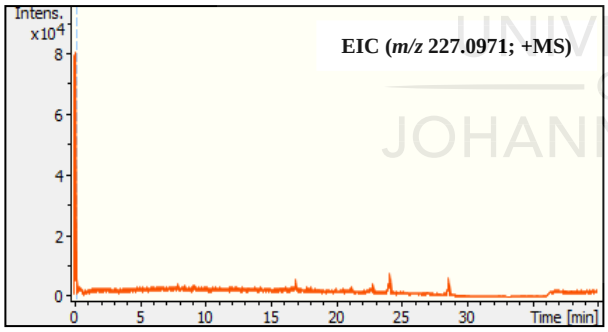
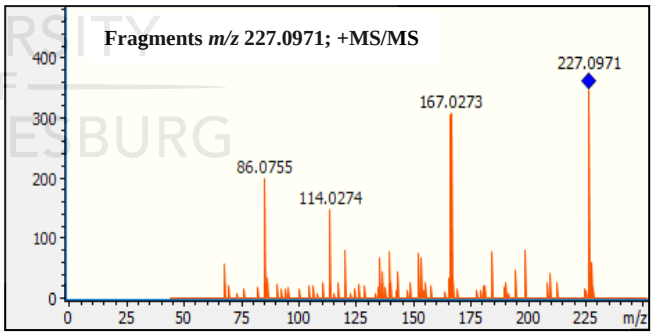
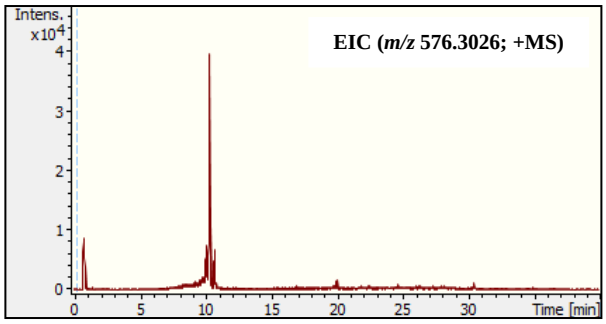
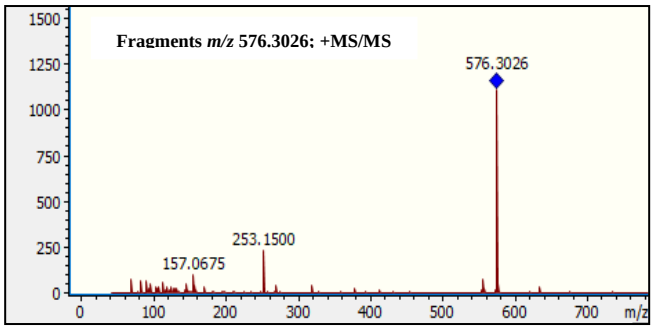
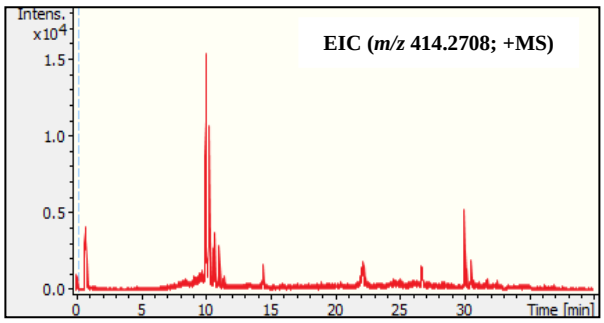
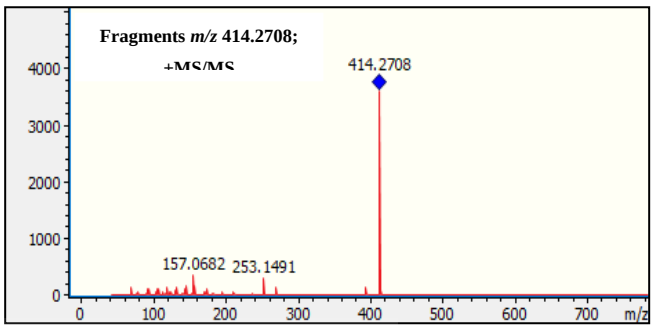
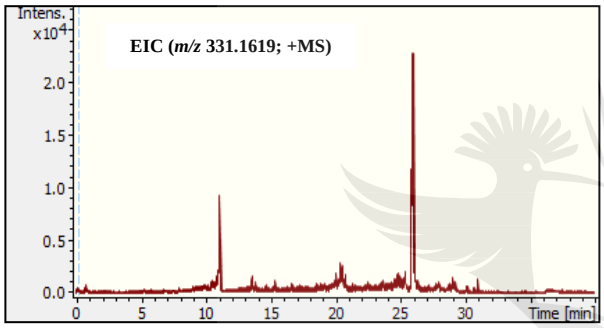
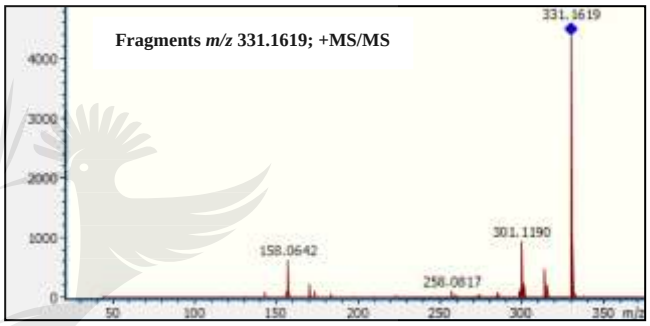
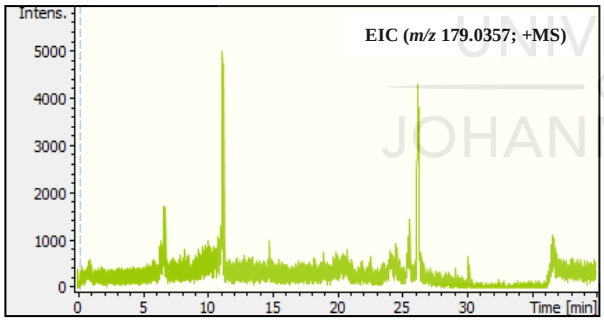
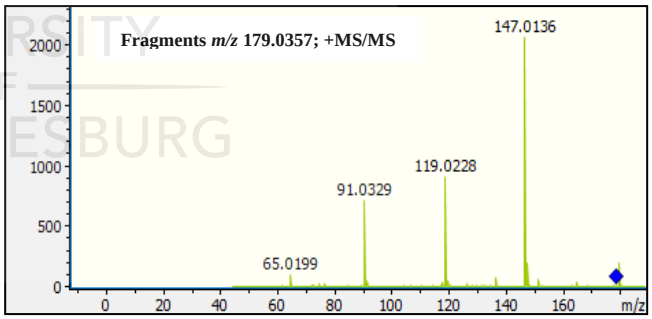
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Tingenone 	

Table A9: Extracted ion chromatogram (EIC) of secondary metabolites from *Solanum mauritianum* ripe fruits seeds – S2 RFS (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Scopoletin	
	
Danielone	
	
Solasonine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
α-Solanine	
	
Imperialine	
	
Genipin	
	
α-Ergocryptine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Solasodine	
	
Podolide	
	
Esculetin	
	
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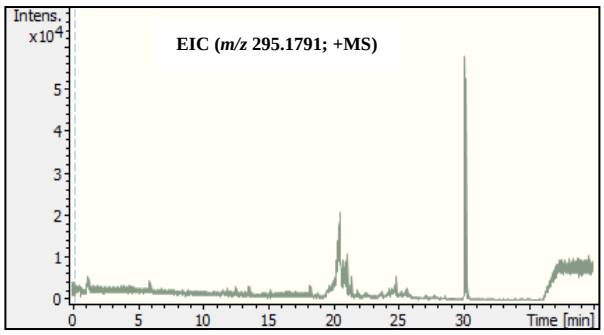
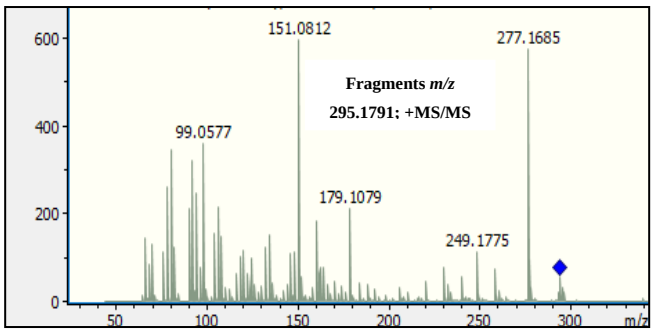
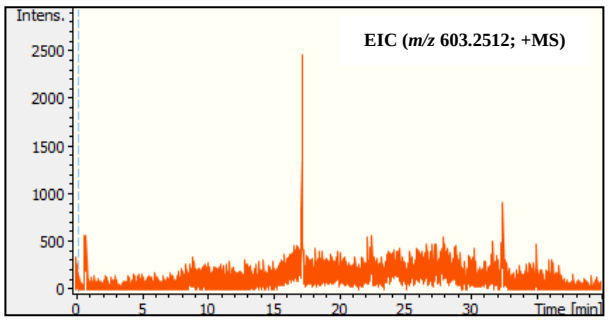
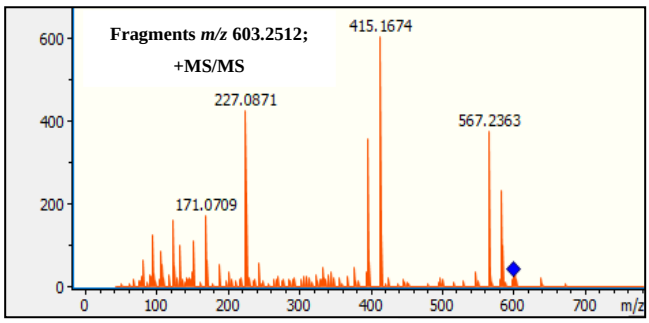
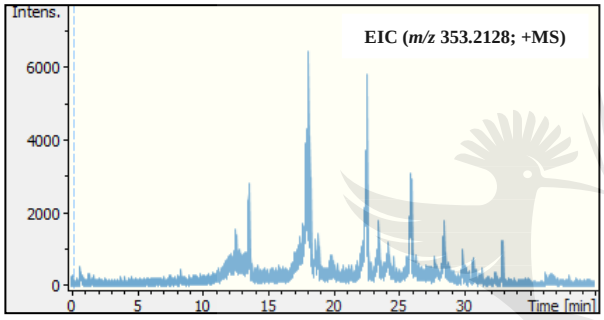
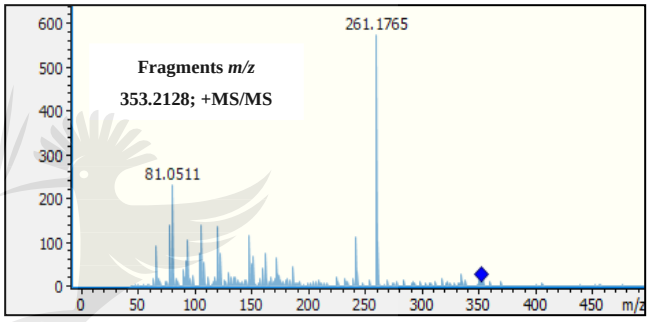
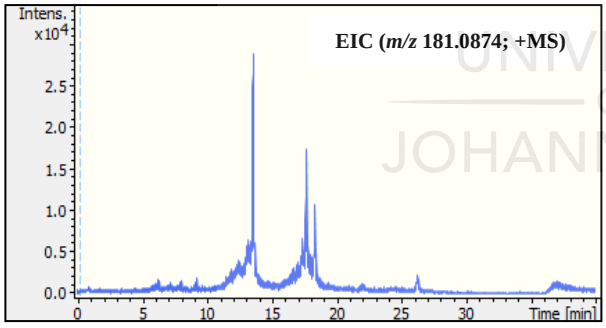
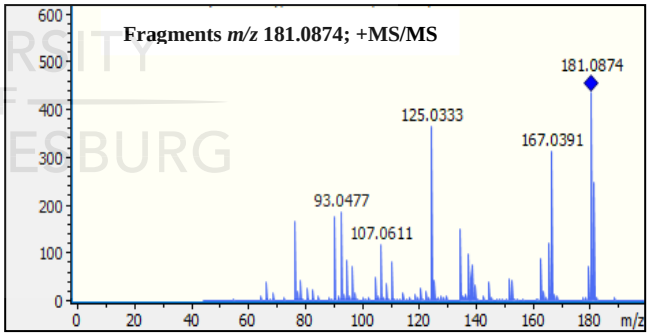
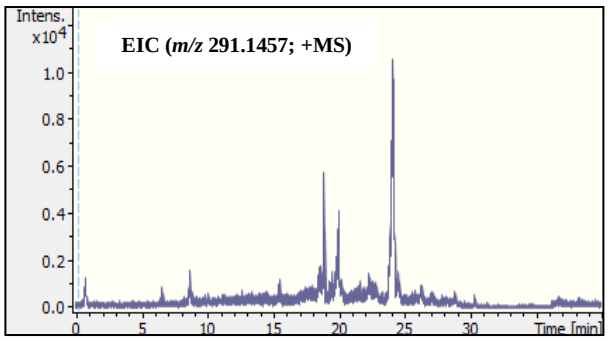
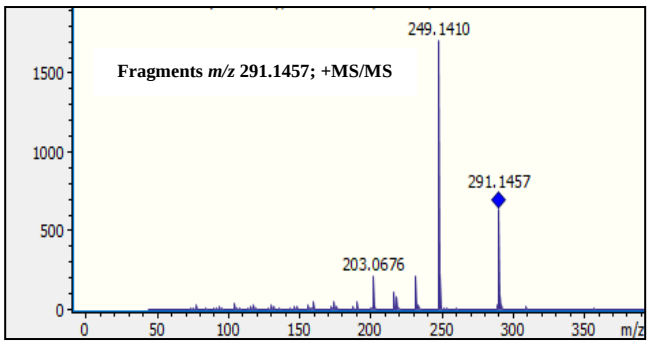
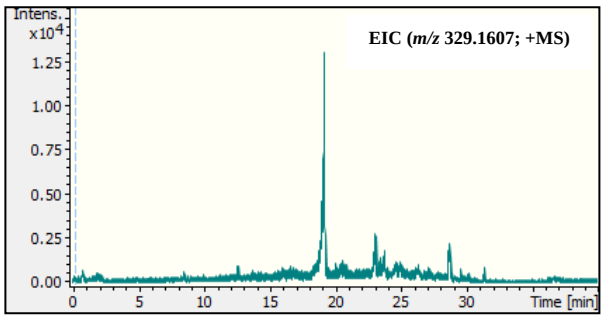
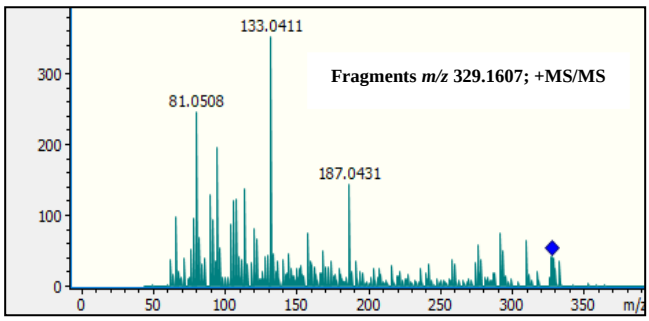
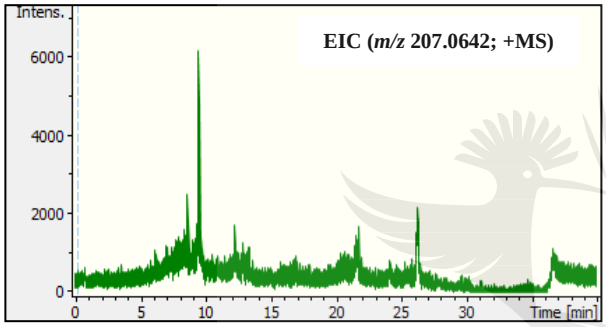
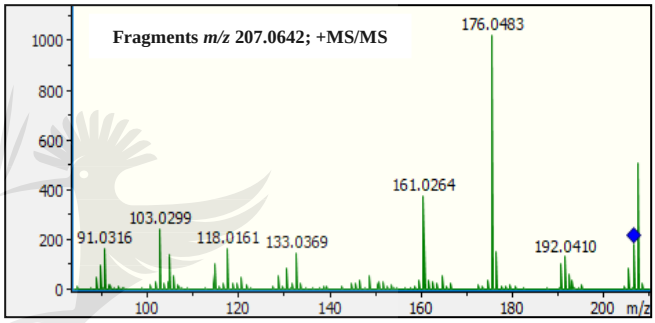
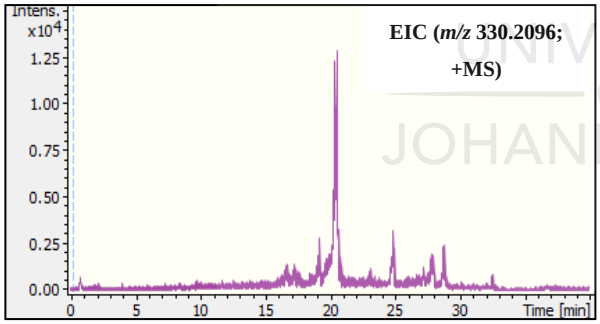
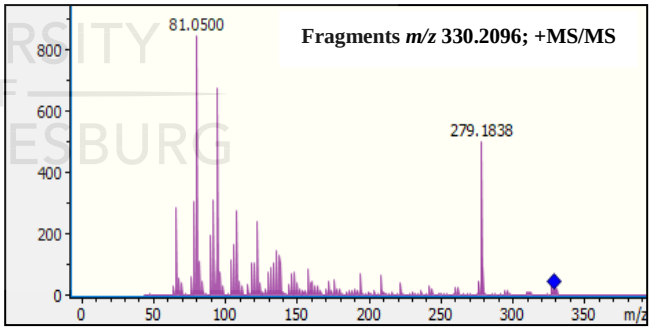
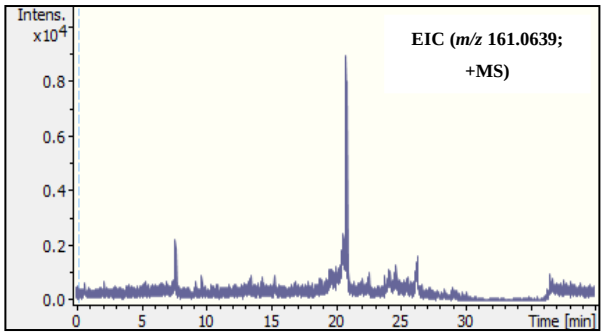
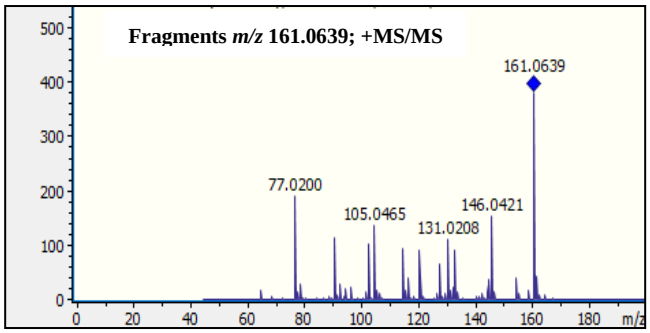
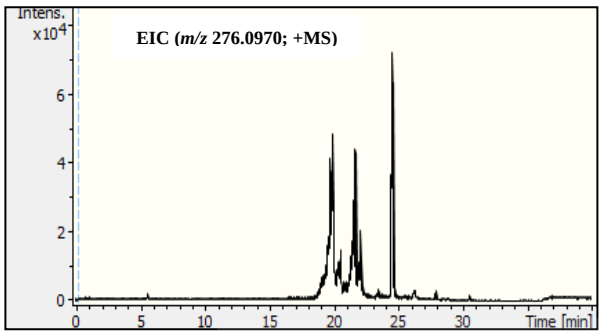
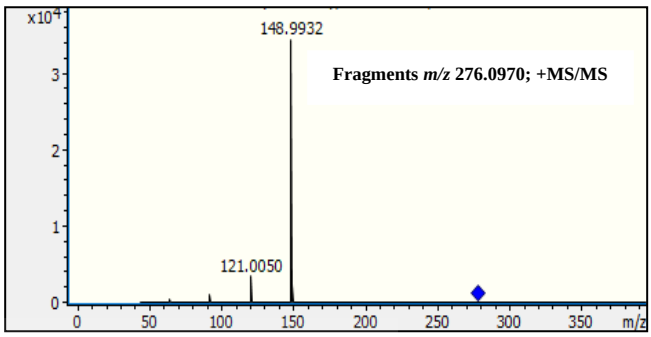
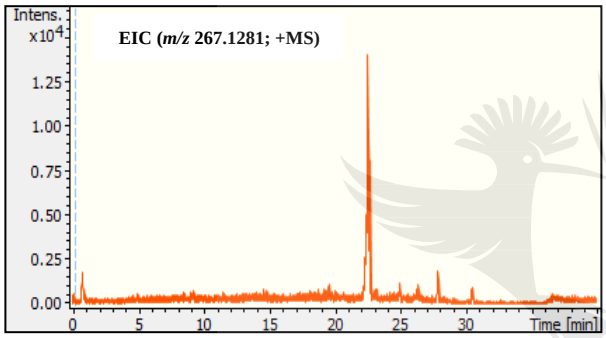
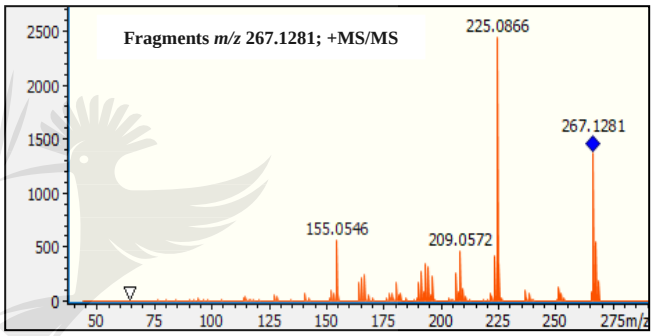
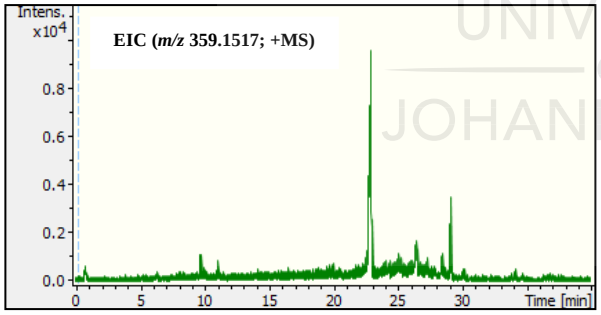
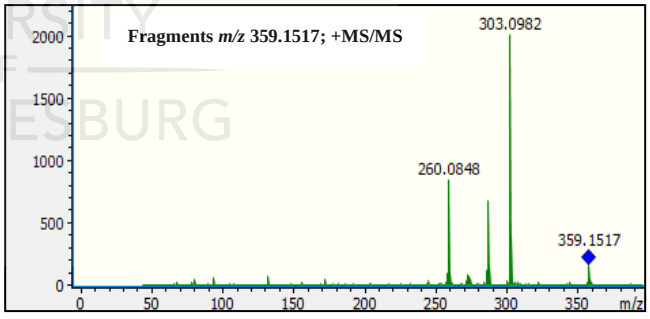
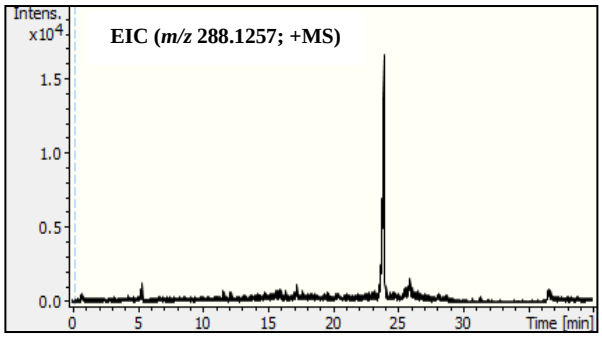
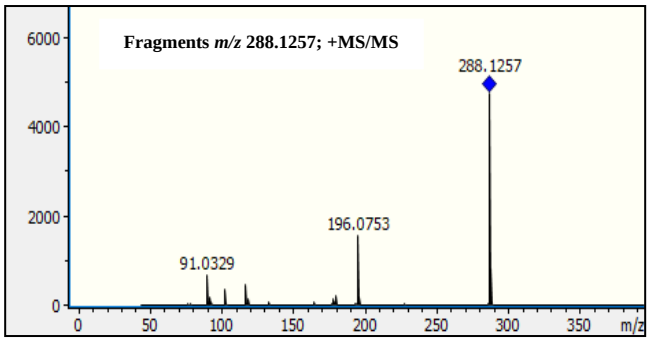
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Glycobismine A	
	
Montanol	
	
Coniferyl alcohol	
	
Ribalinium	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Rhododendrin	
	
Eugenin	
	
Hetisine	
	
Anatabine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Tuliposide A	
	
Vasconine	
	
Columbin	
	
Lycorine	
	

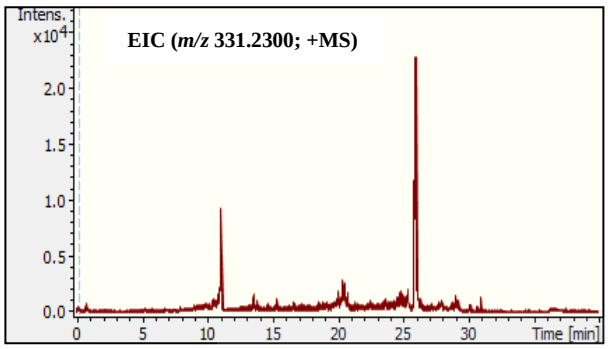
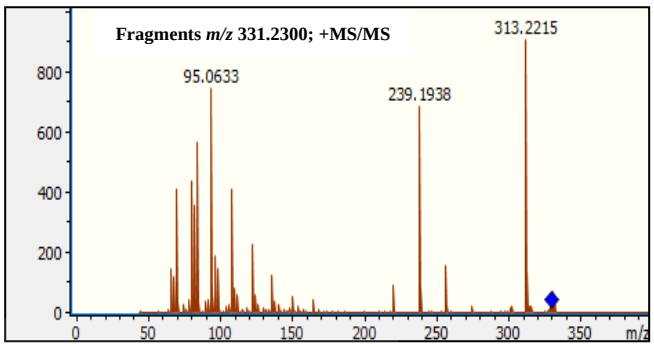
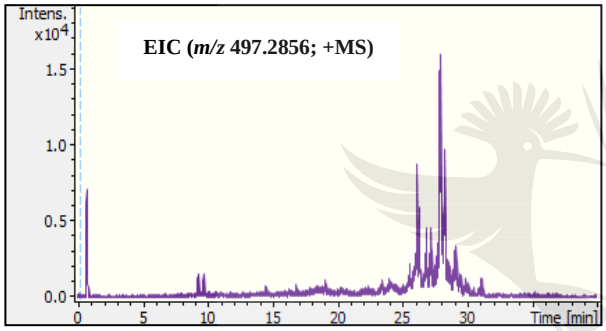
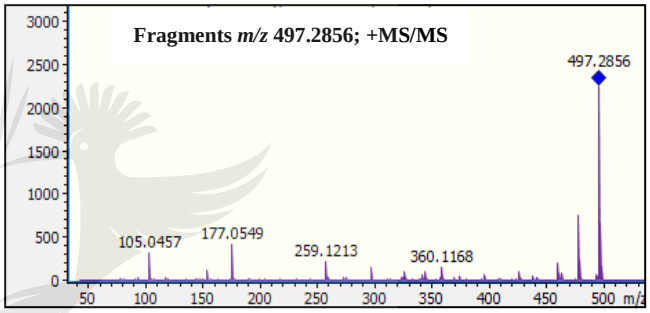
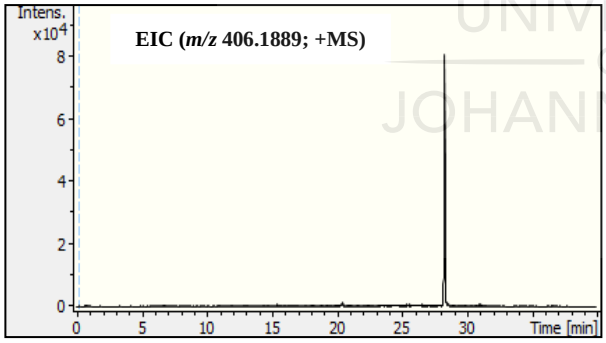
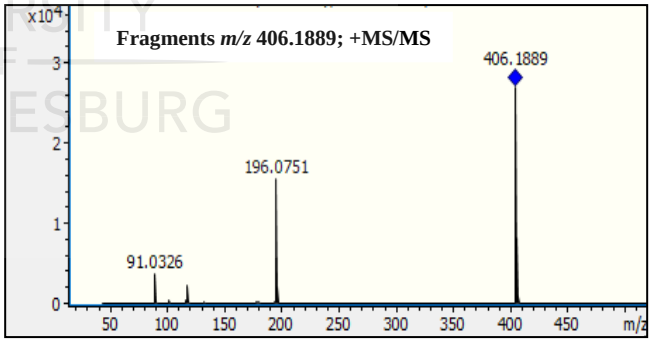
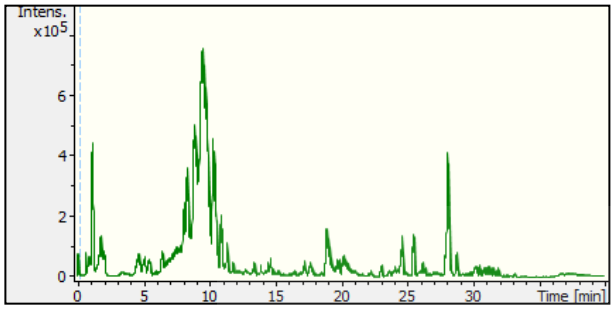
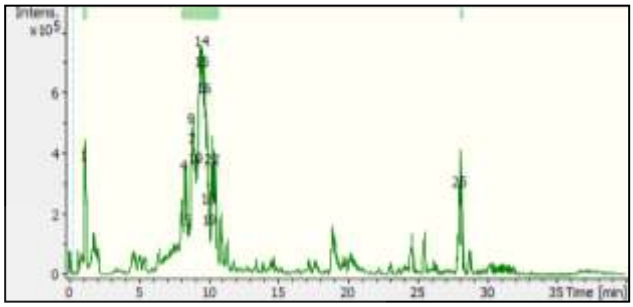
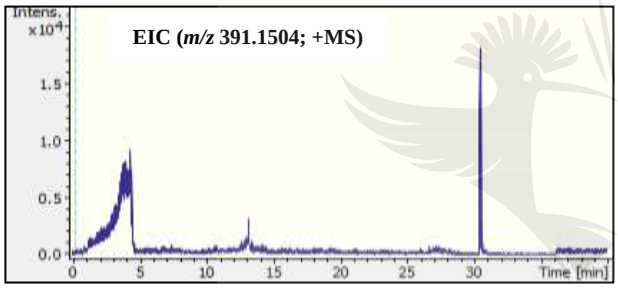
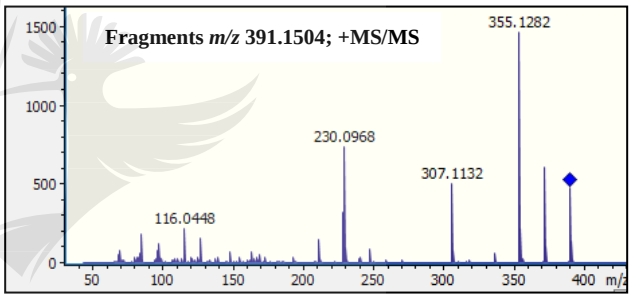
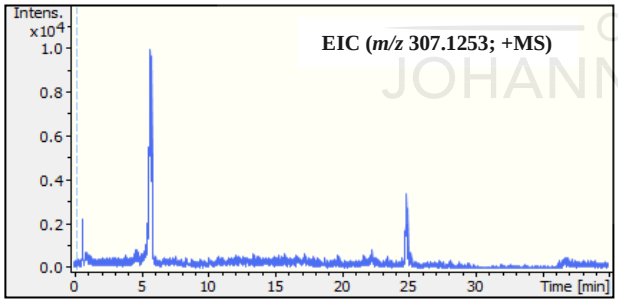
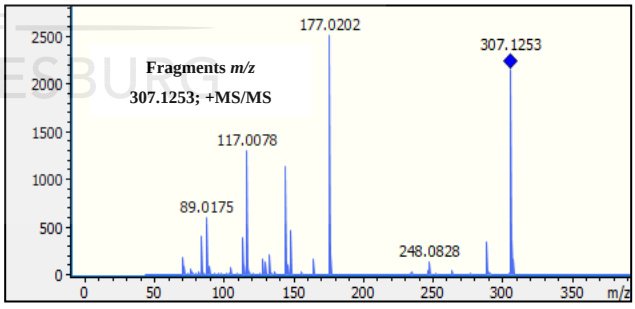
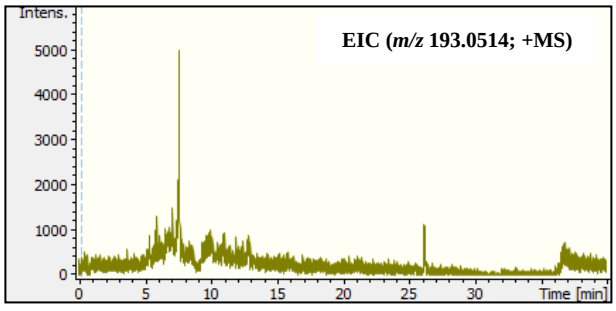
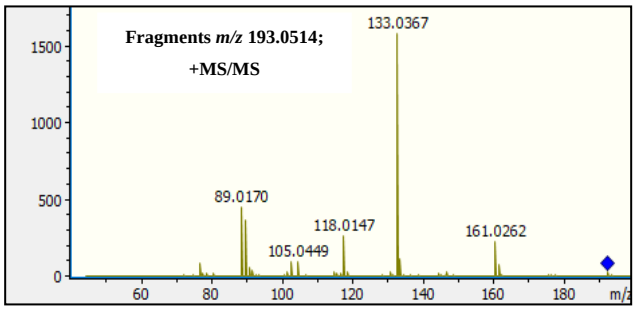
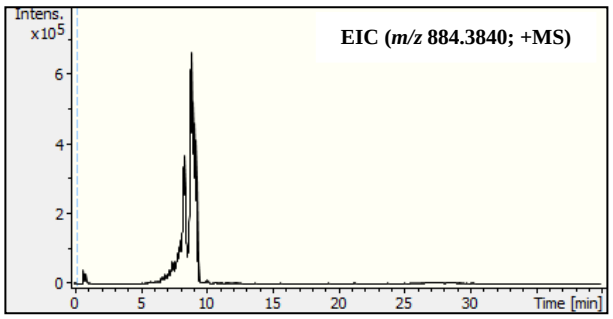
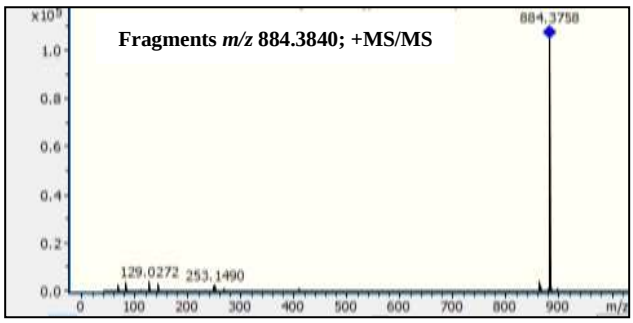
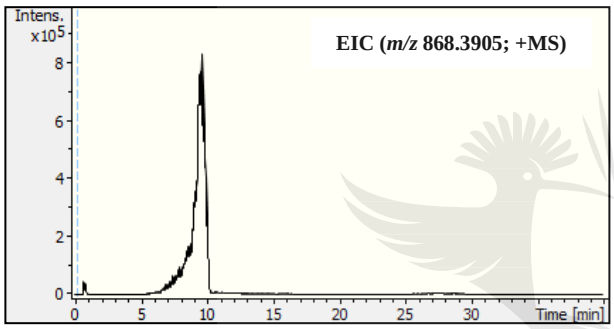
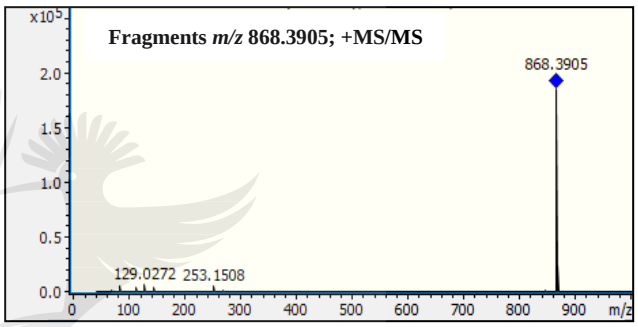
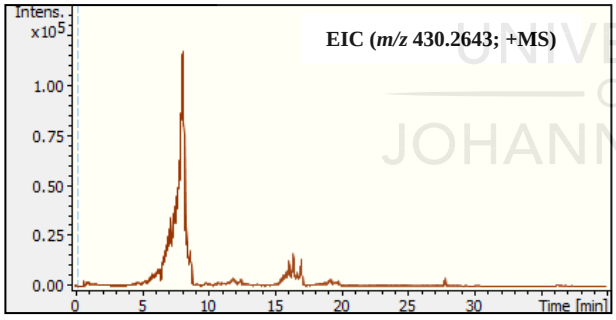
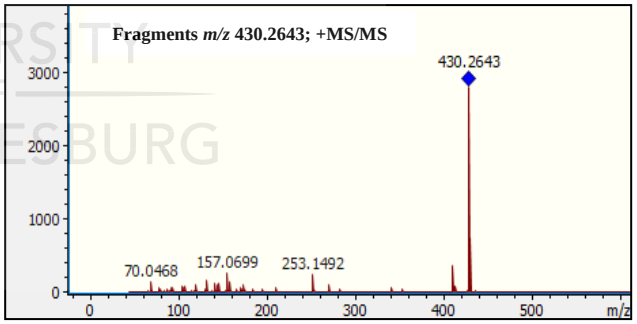
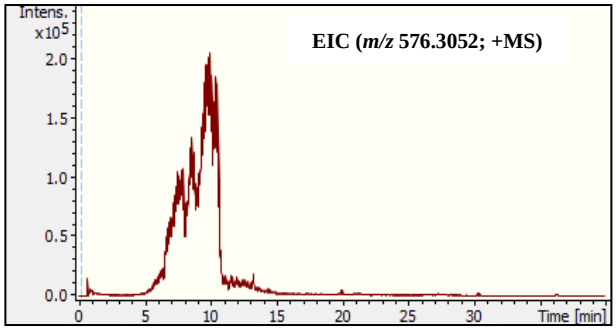
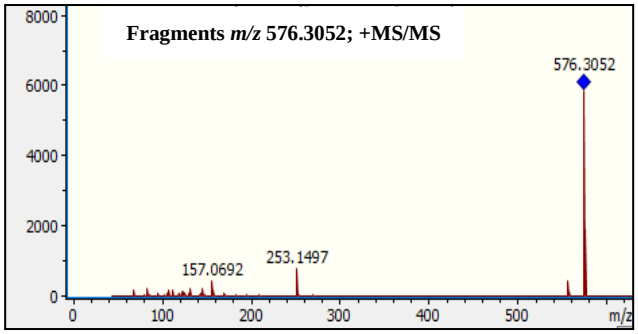
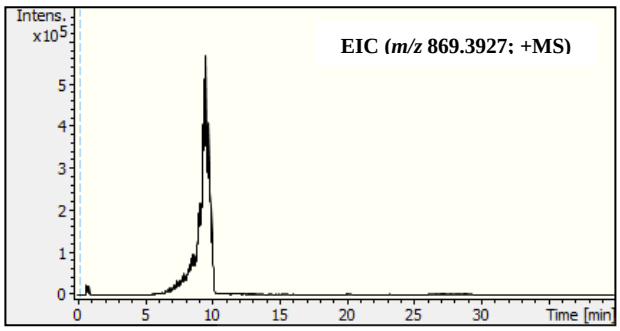
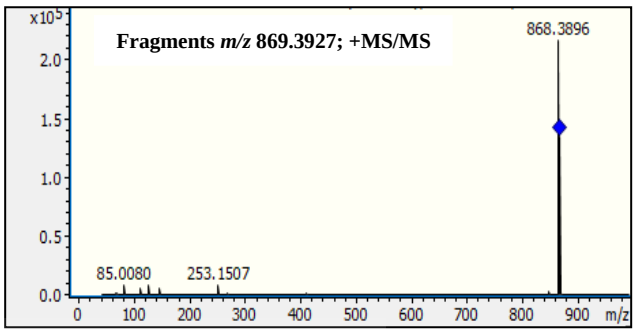
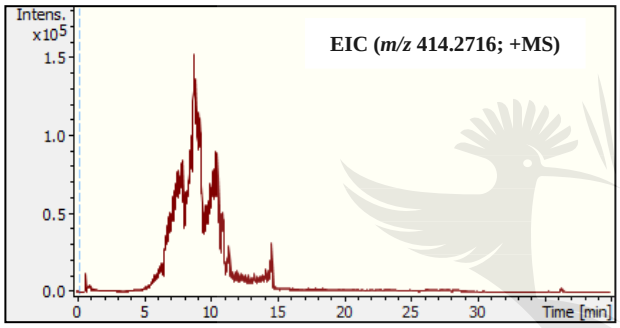
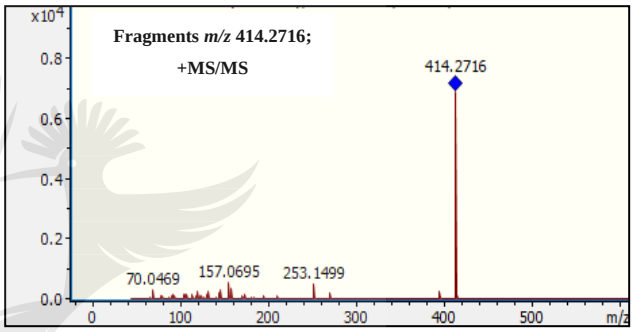
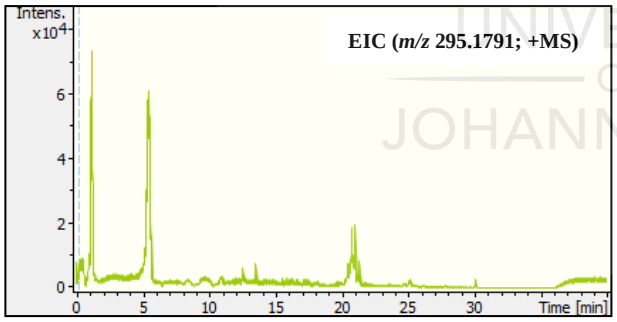
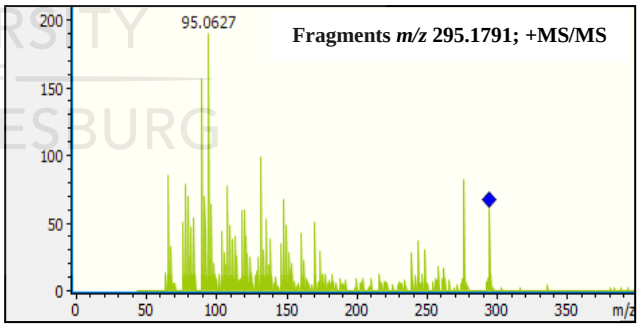
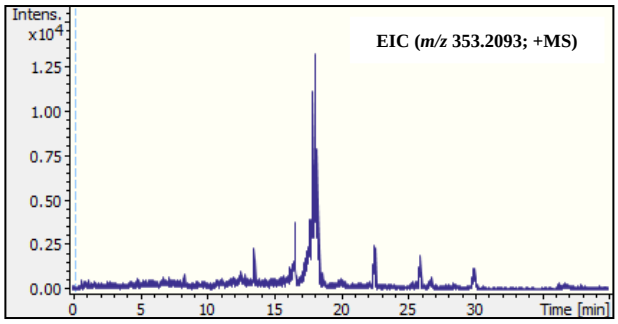
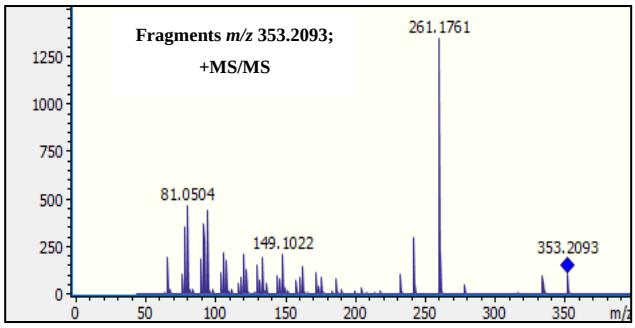
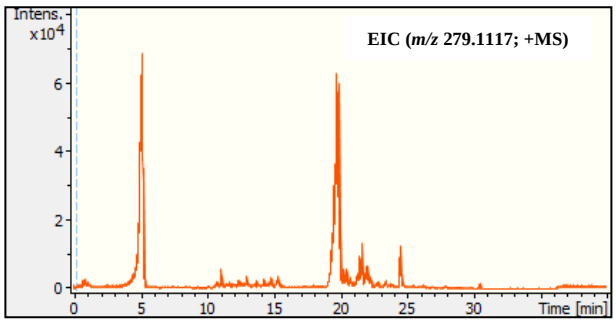
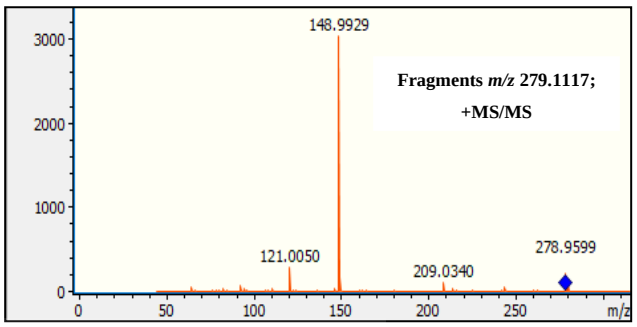
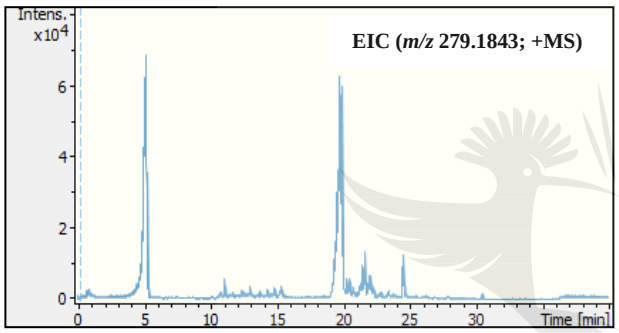
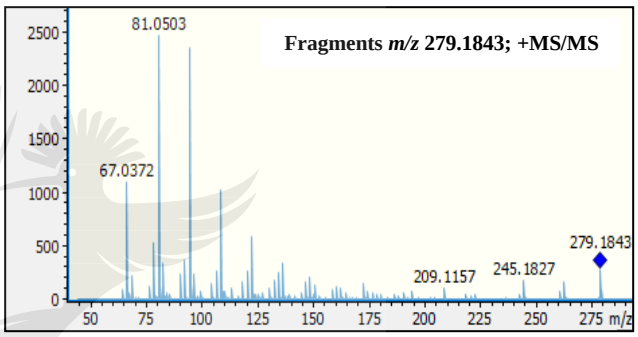
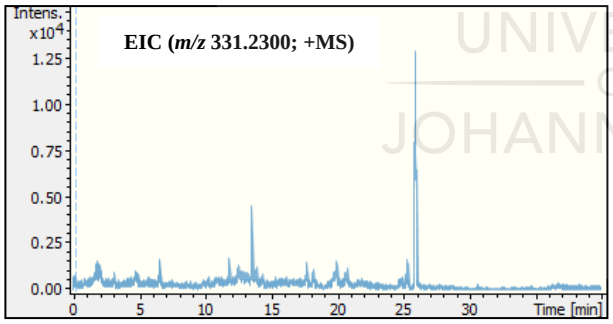
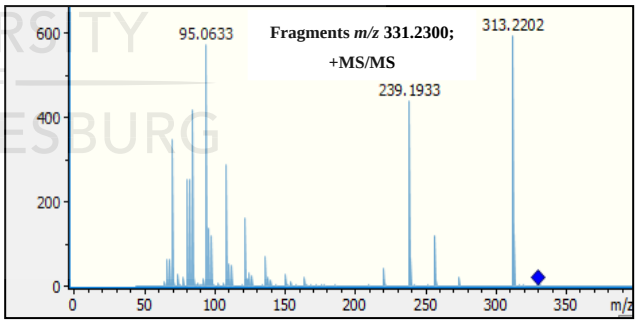
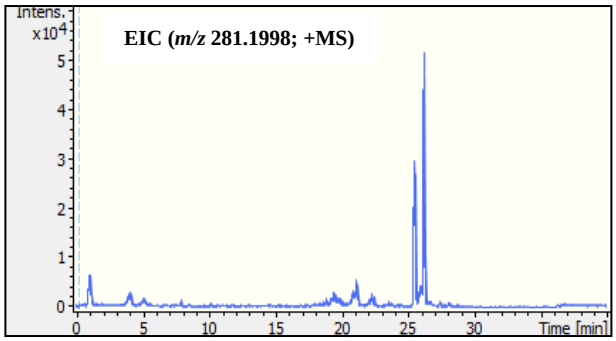
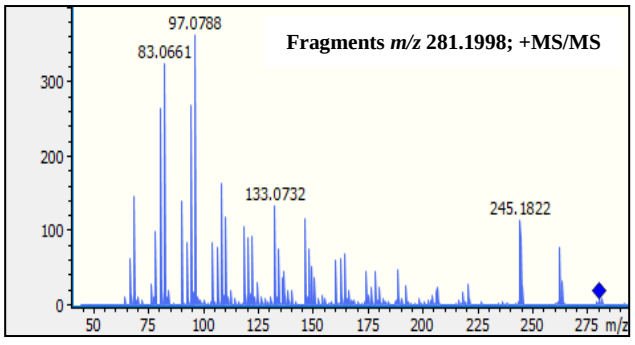
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Cannabielsoin	
	
Absinthin	
	
Clivoline	
	

Table A10: Extracted ion chromatogram (EIC) of secondary metabolites from *Solanum mauritianum* unripe (green) fruits seeds – S3 GFS (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC) 	
Loganin 	
Cimifugin 	
Scopoletin 	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Solasonine	
	
α-Solanine	
	
Imperialine	
	
α-Ergocryptine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Dioscin	
	
Solasodine	
	
10-Deoxysarpagine	
	
Montanol	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Tuliposide A	
	
Lycocernuine	
	
Cannabielsoin	
	
Ibogamine	
	

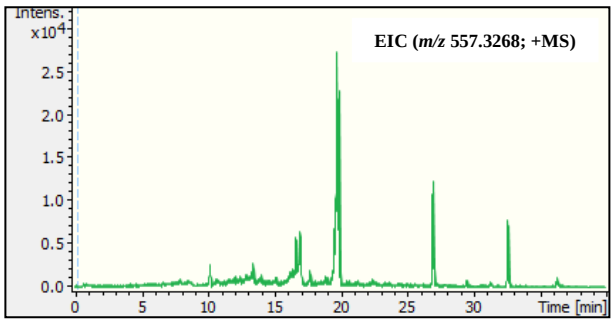
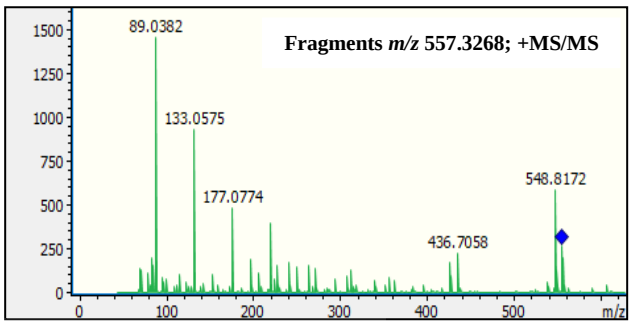
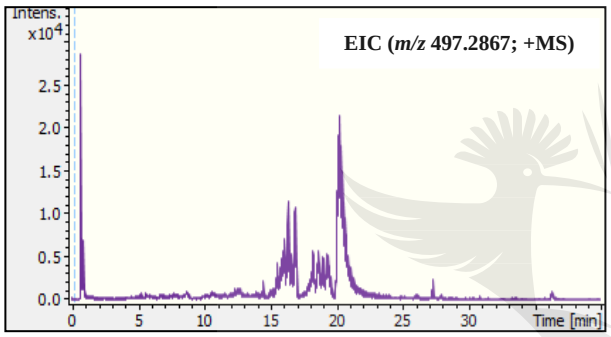
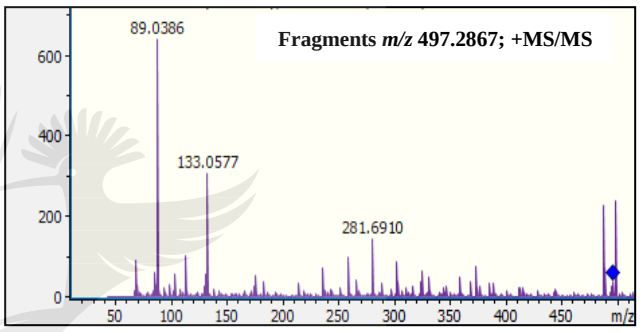
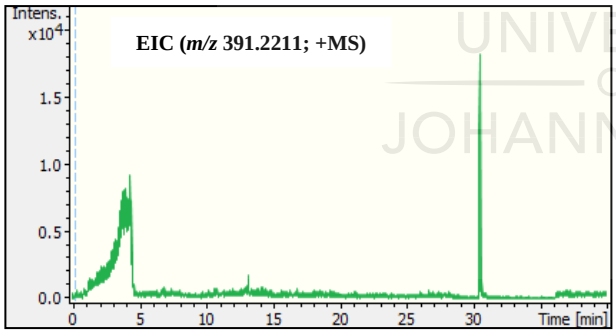
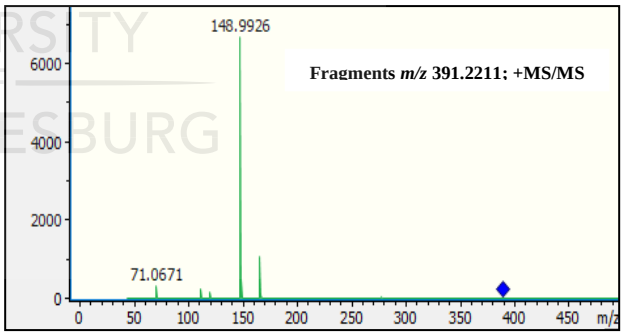
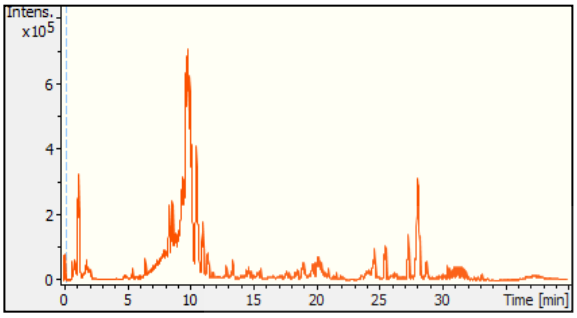
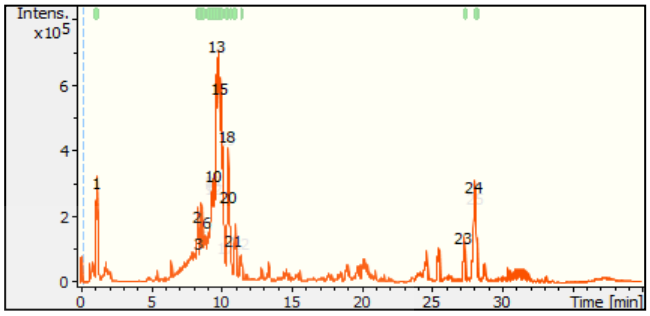
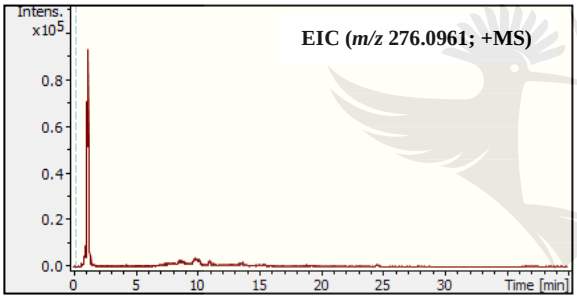
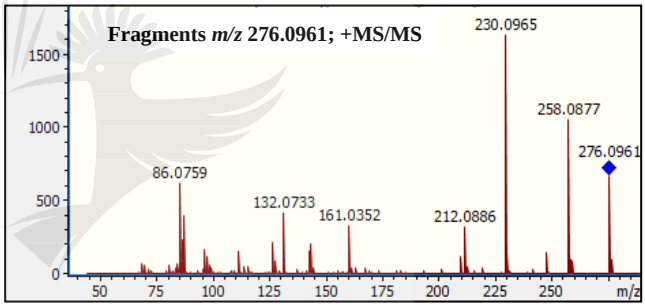
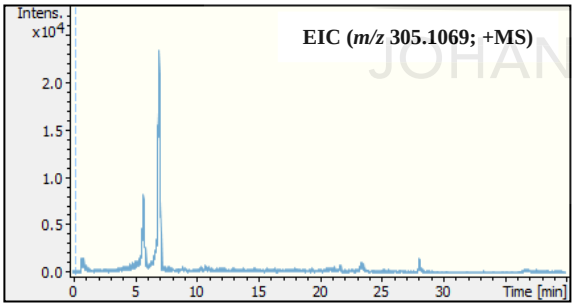
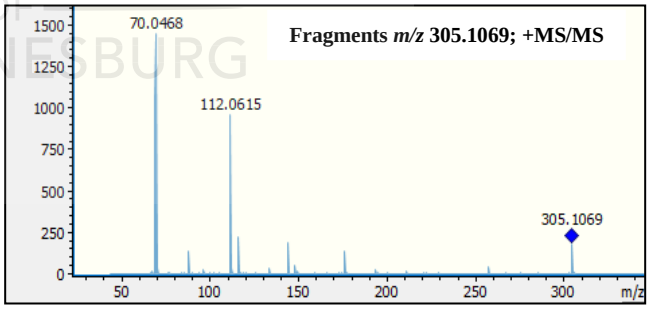
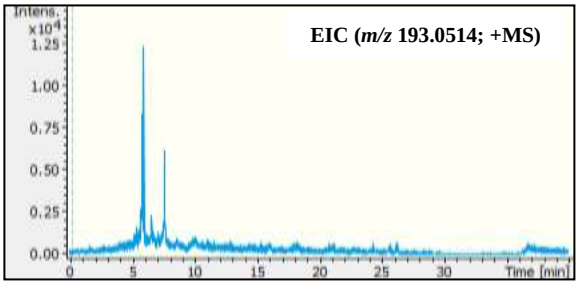
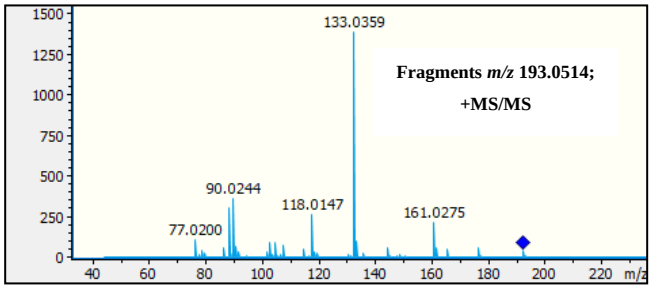
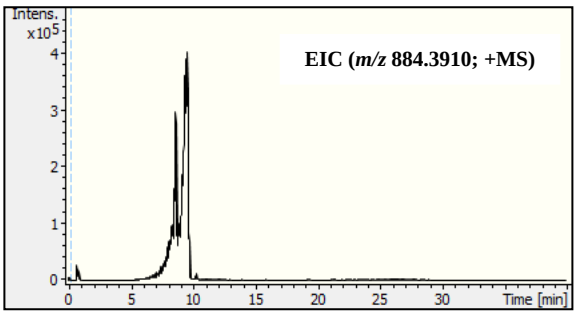
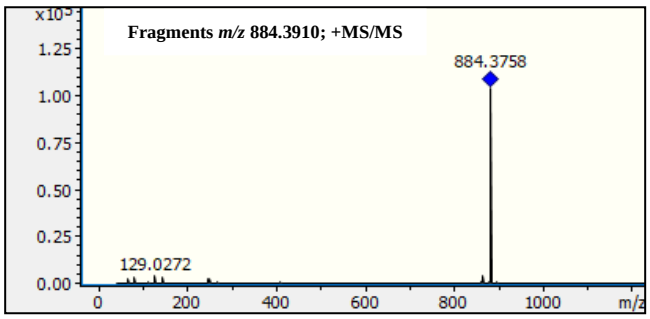
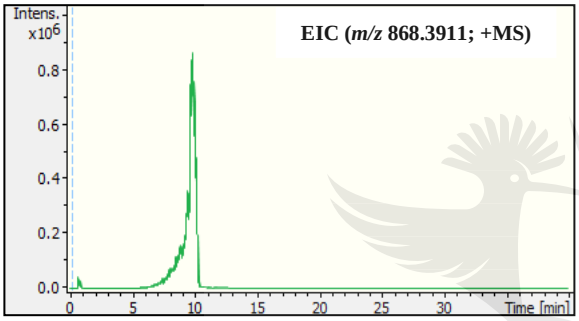
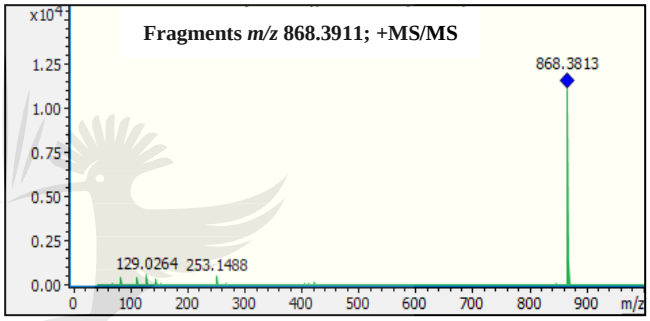
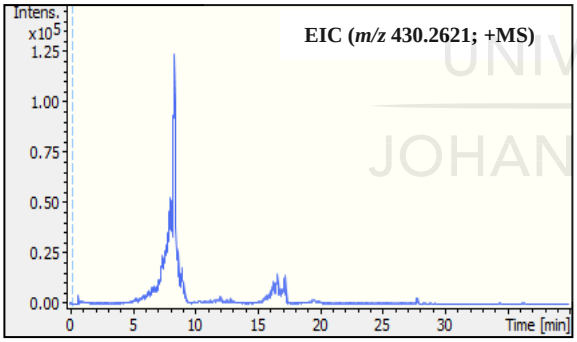
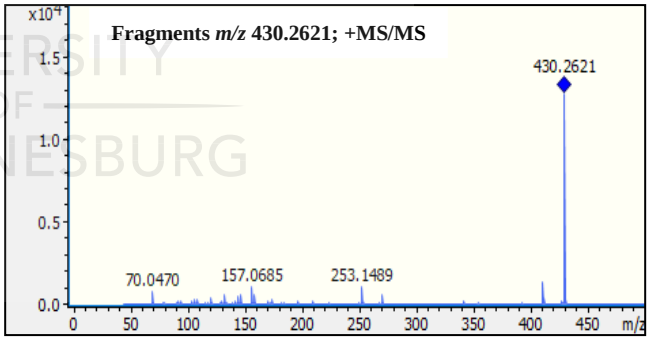
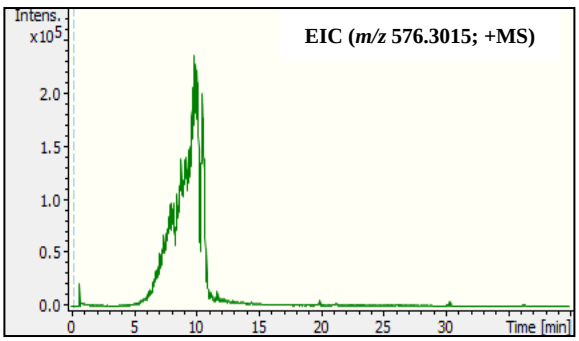
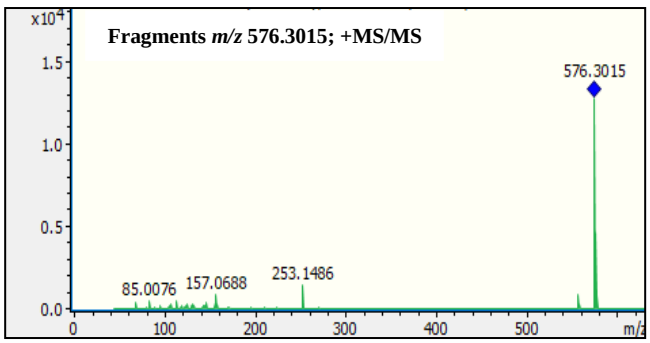
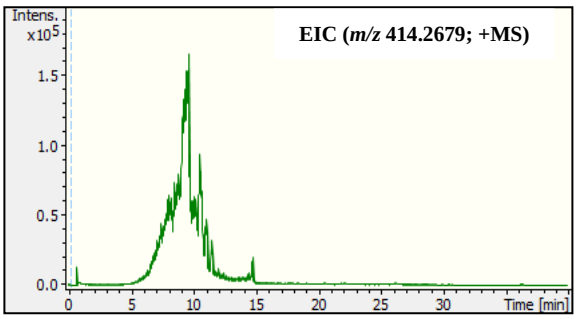
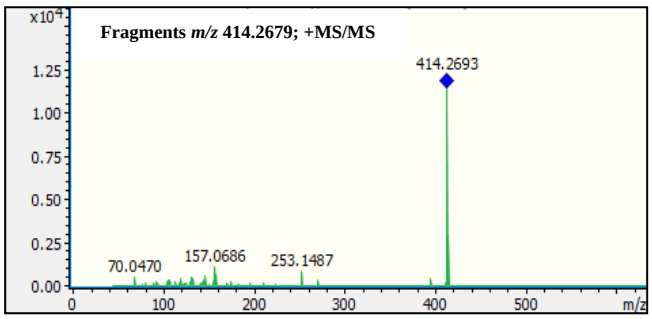
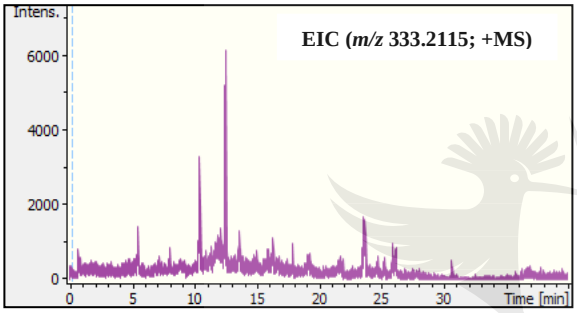
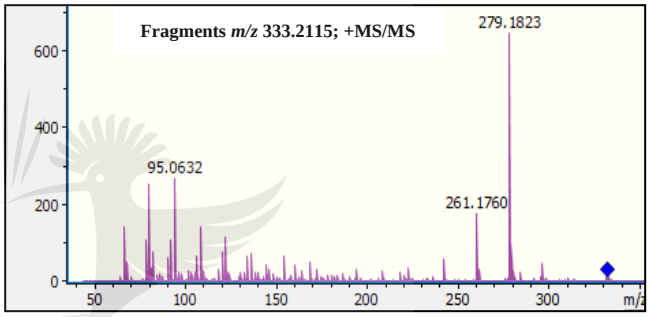
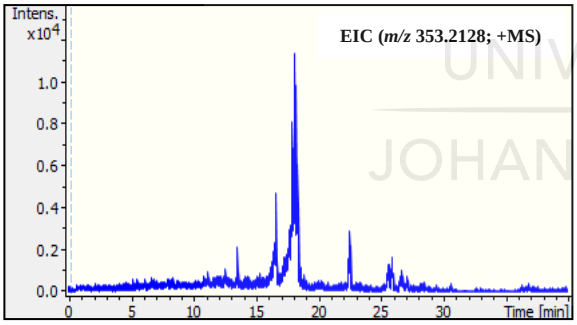
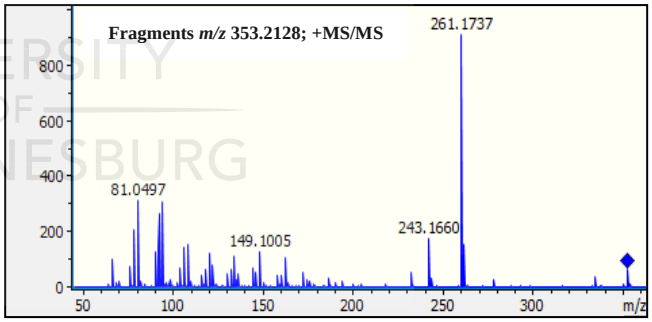
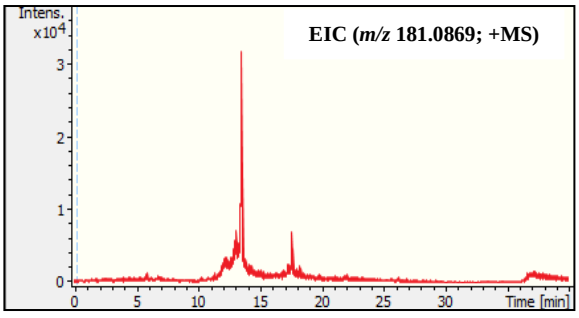
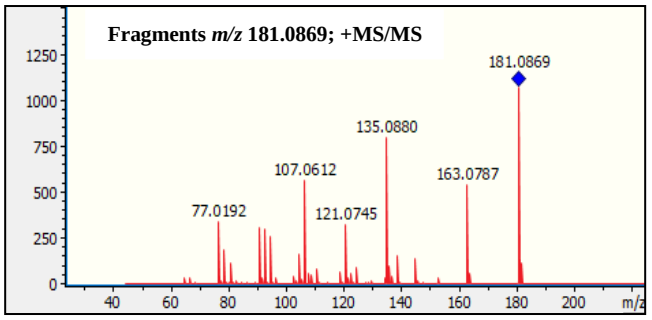
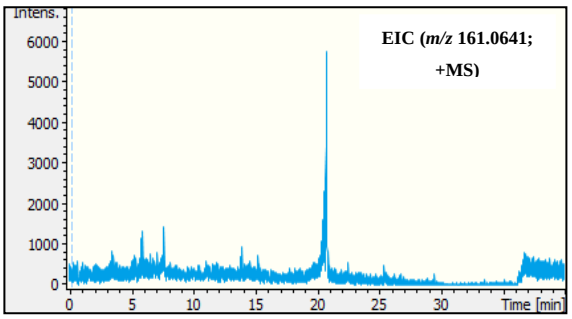
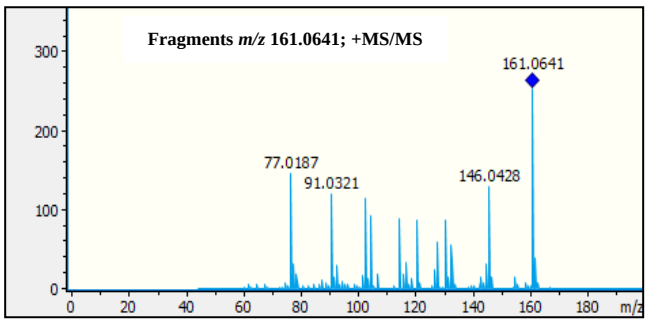
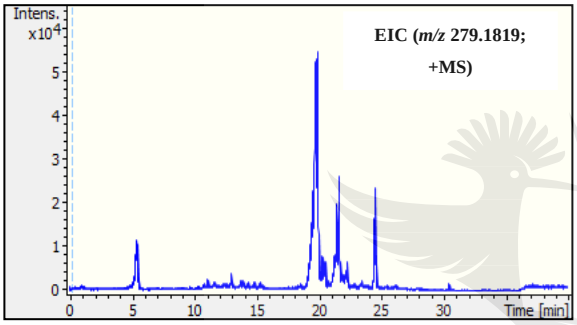
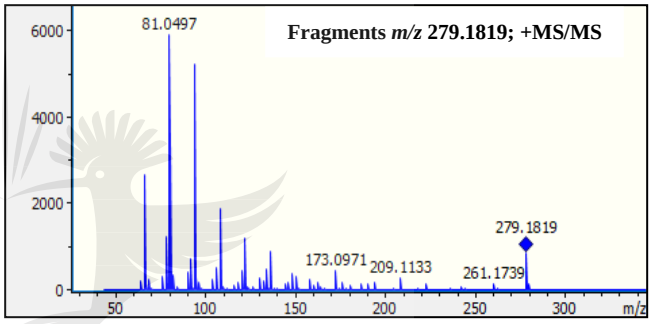
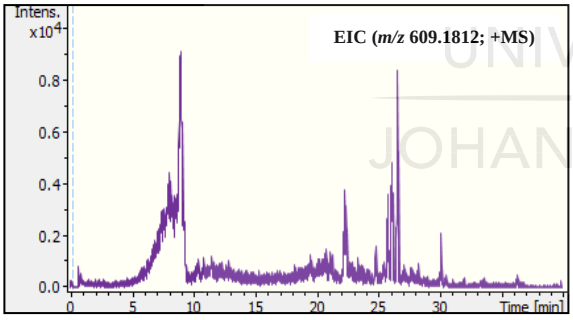
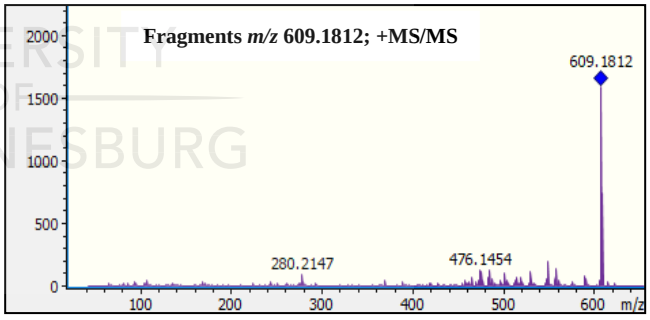
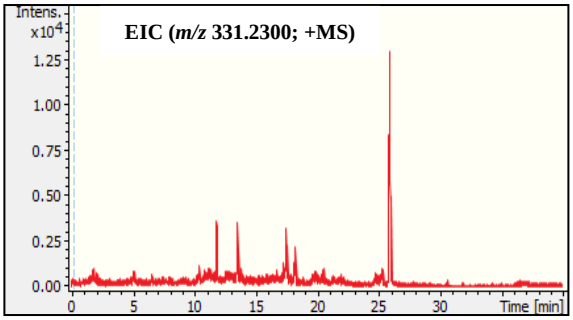
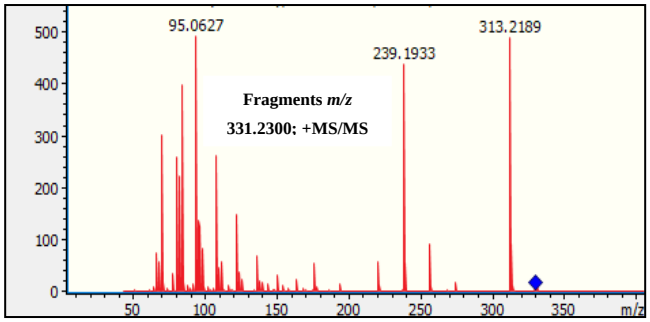
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Cucurbitacin E	
	
Absinthin	
	
Neoquassin	
	

Table A11: Extracted ion chromatogram (EIC) of secondary metabolites from *Solanum mauritianum* unripe (green) fruits coat – S4 GFC (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC) 	
Cardiospermin 	
Vicine 	
Scopoletin 	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Solasonine	
	
α-Solanine	
	
Imperialine	
	
α-Ergocryptine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Solasodine	
	
Callicarpone	
	
Montanol	
	
Coniferyl alcohol	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Anatabine	
	
Lycocernuine	
	
Diosmin	
	
Cannabielsoin	
	

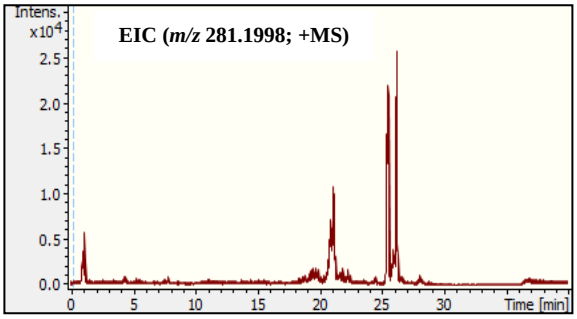
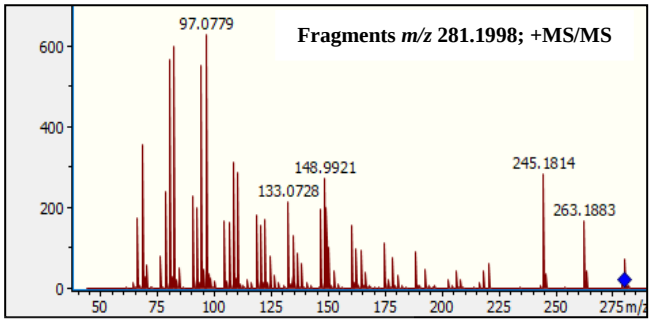
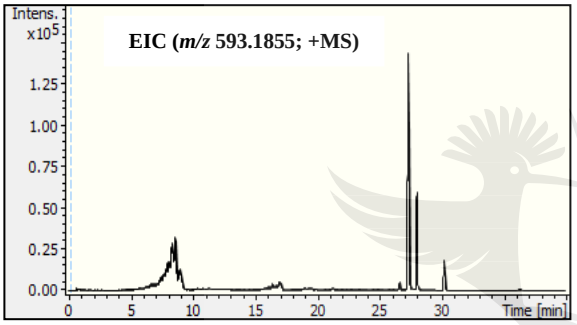
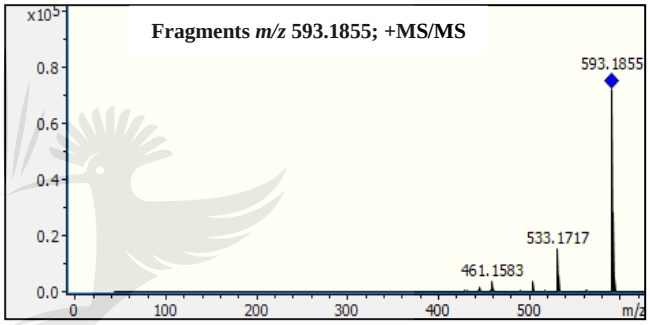
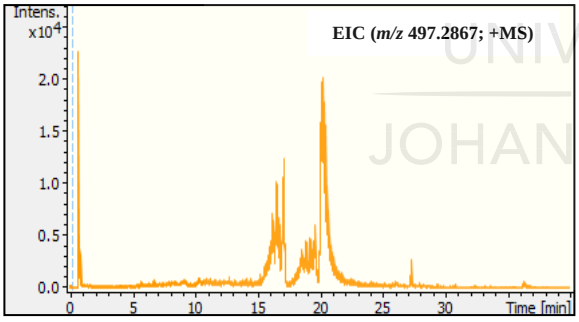
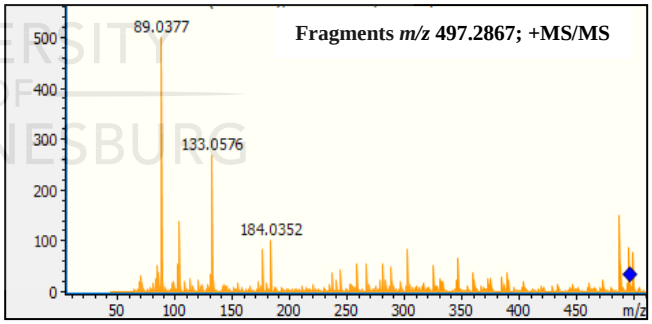
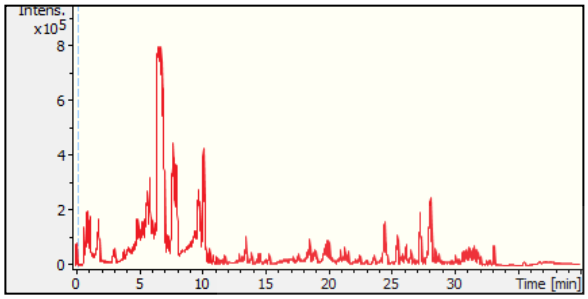
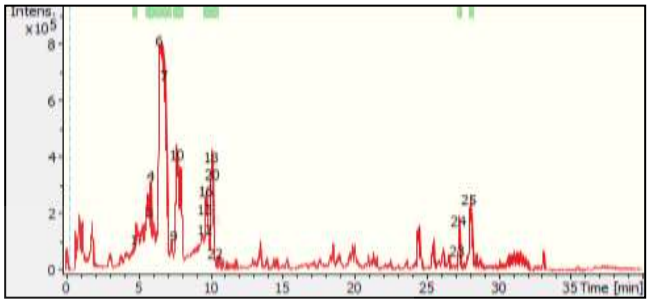
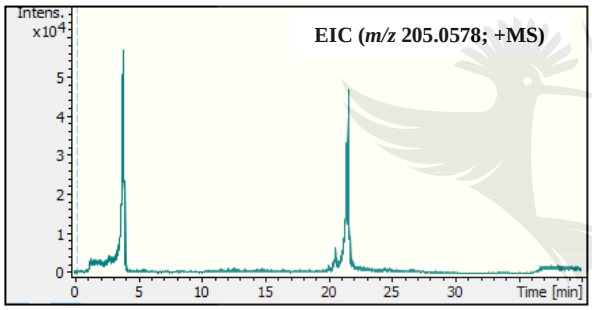
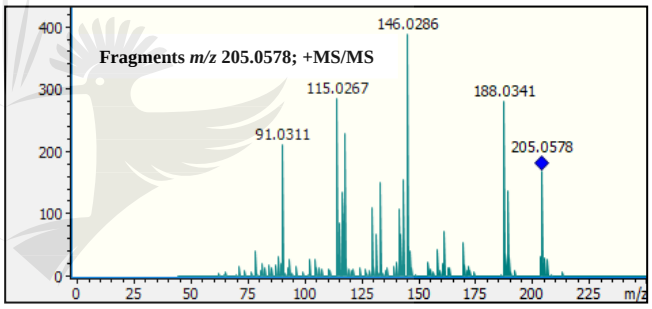
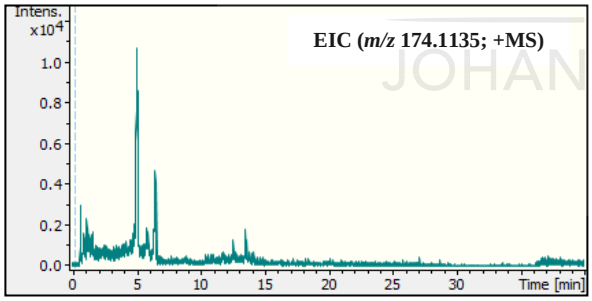
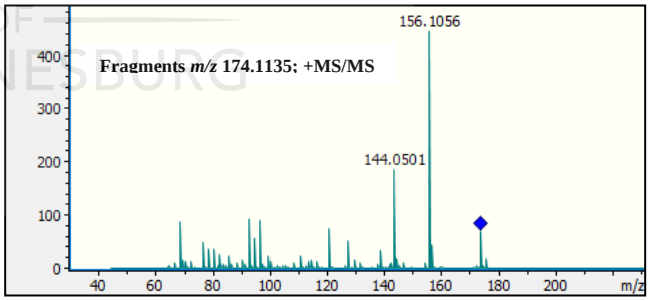
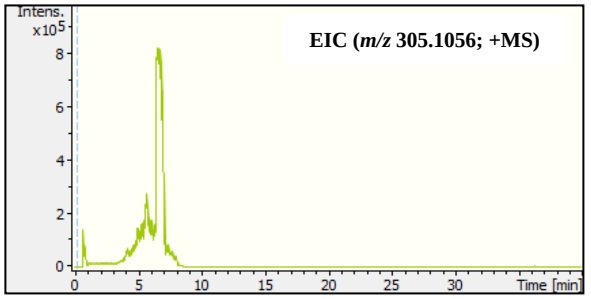
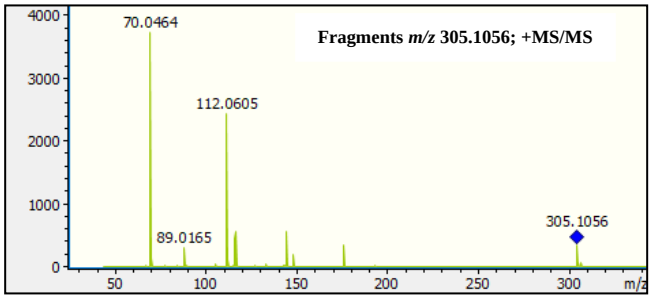
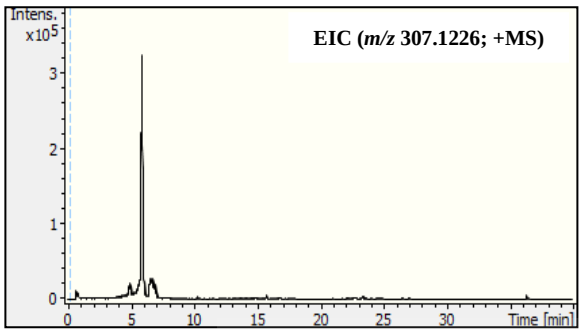
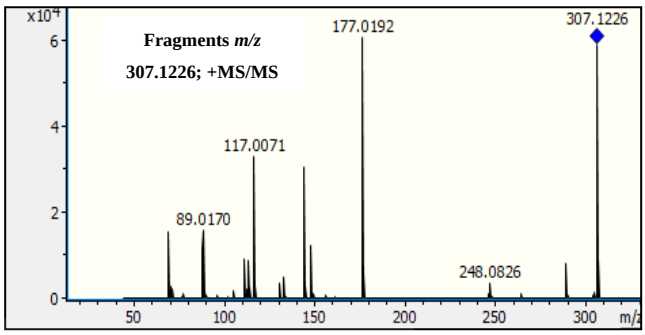
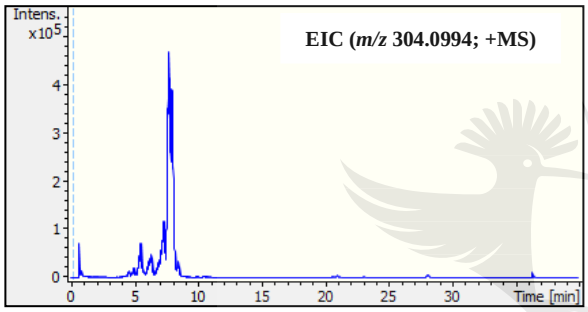
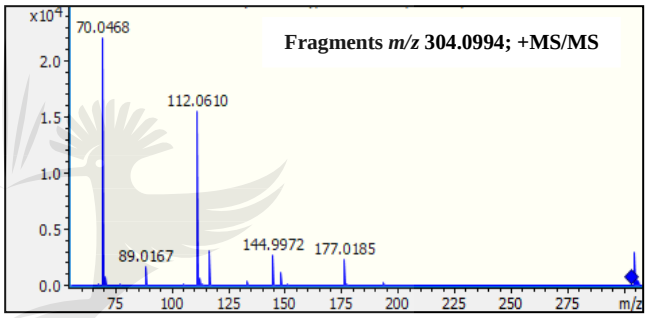
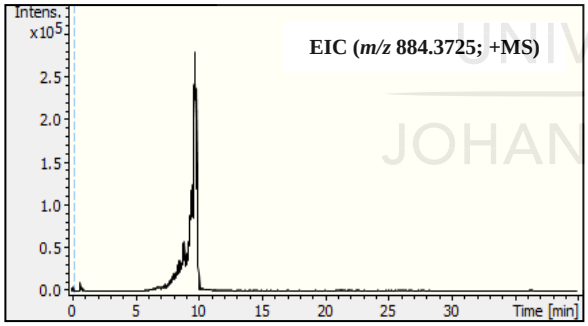
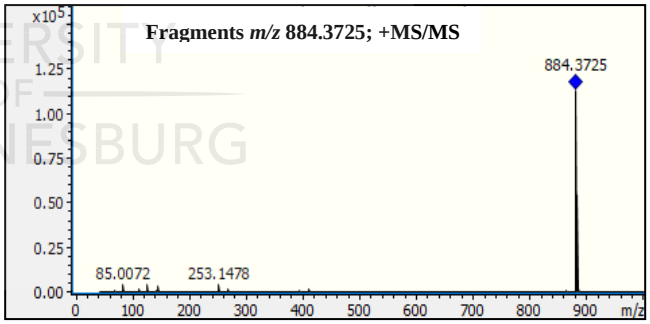
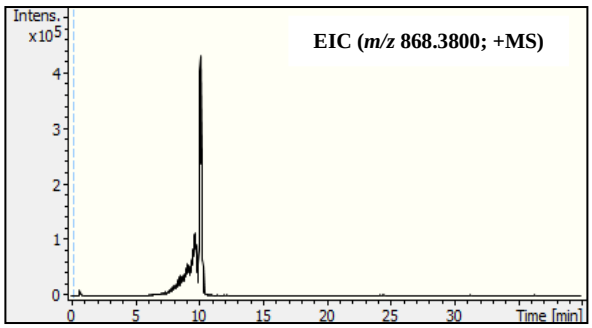
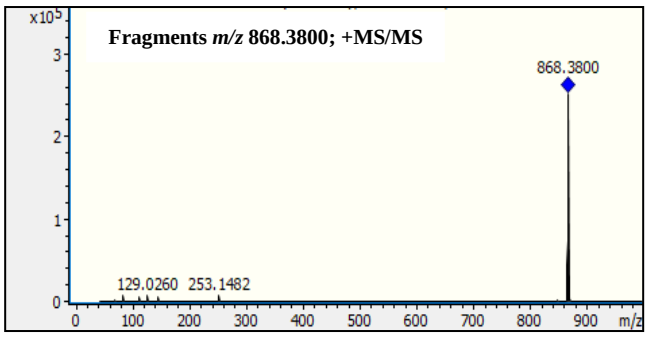
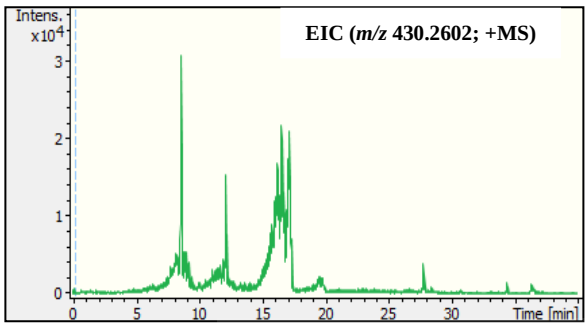
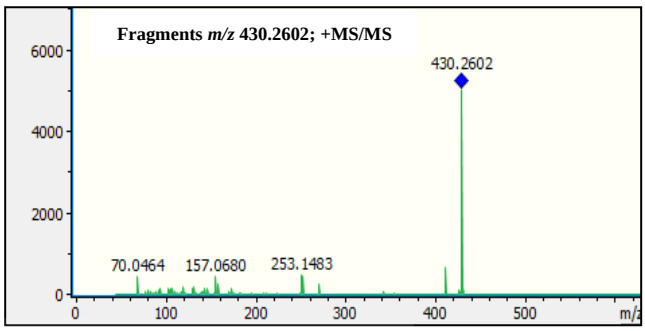
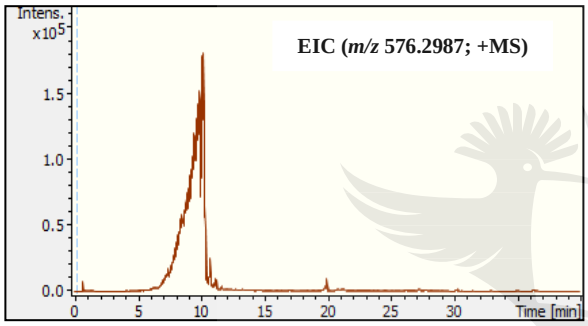
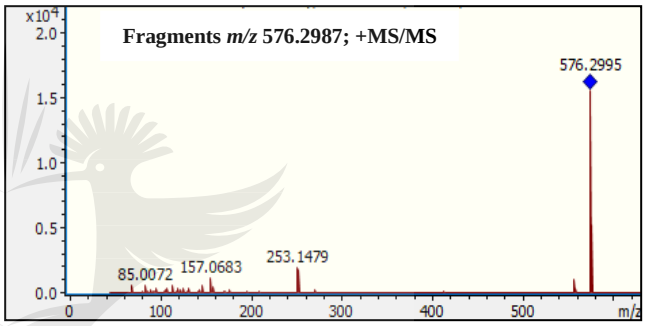
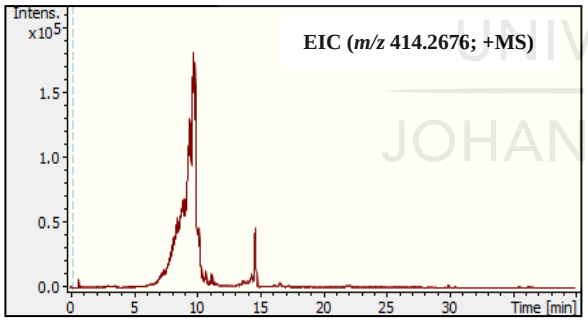
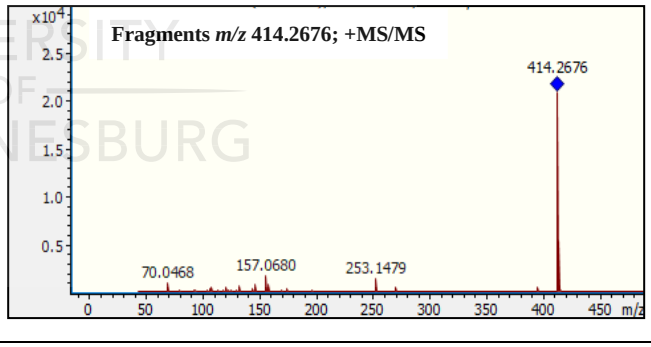
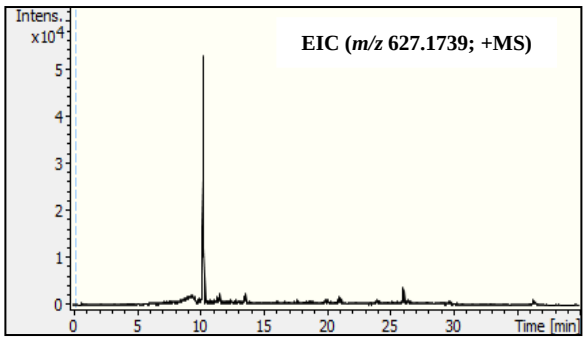
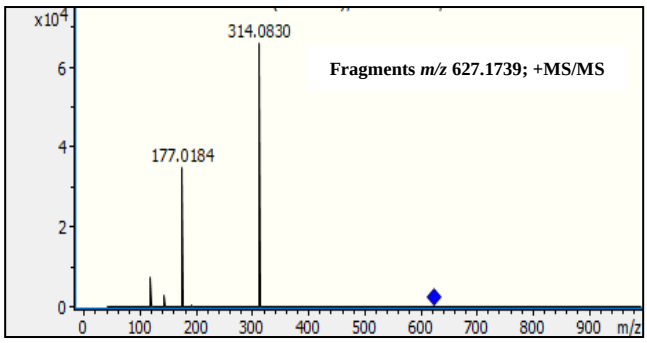
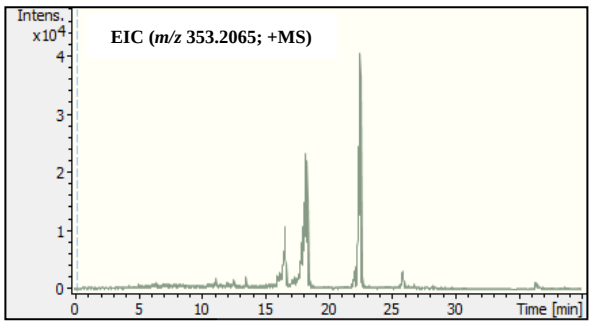
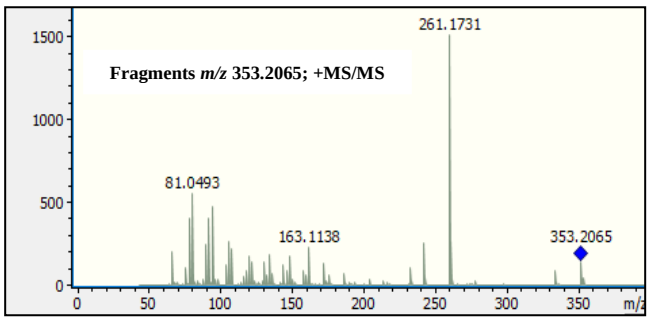
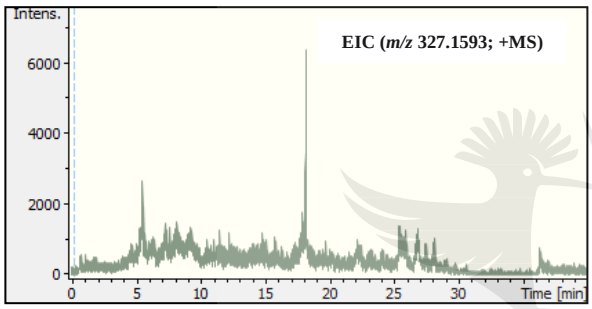
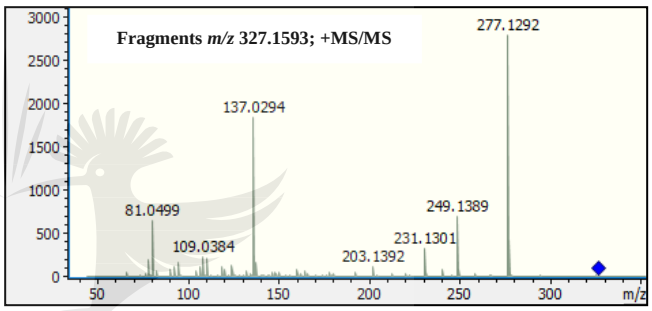
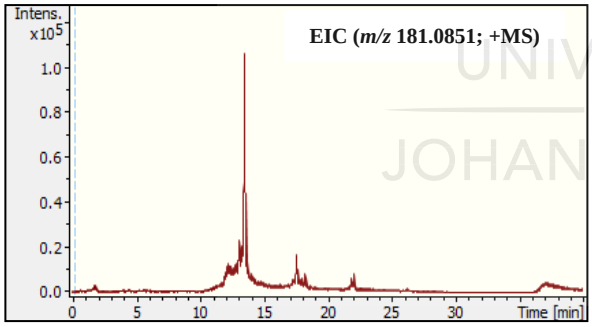
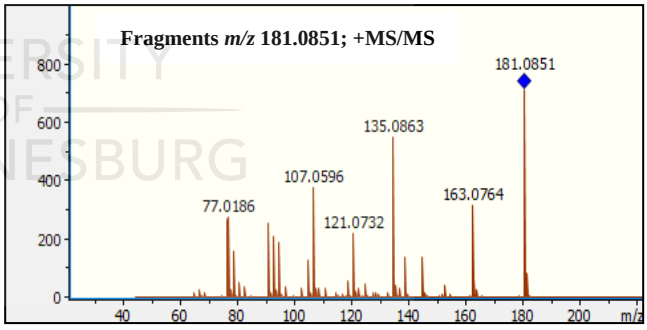
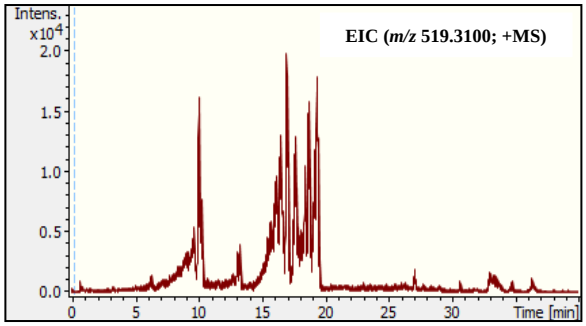
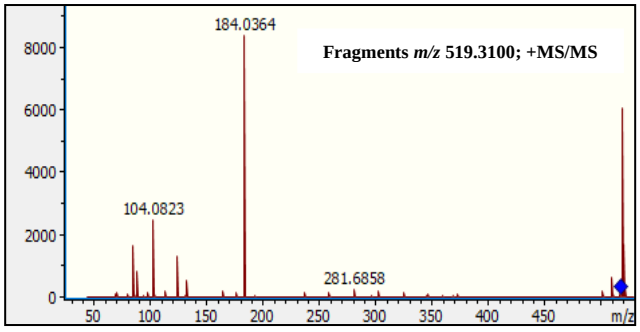
EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Ibogamine	
	
Isoswertisin 2''-rhamnoside	
	
Absinthin	
	

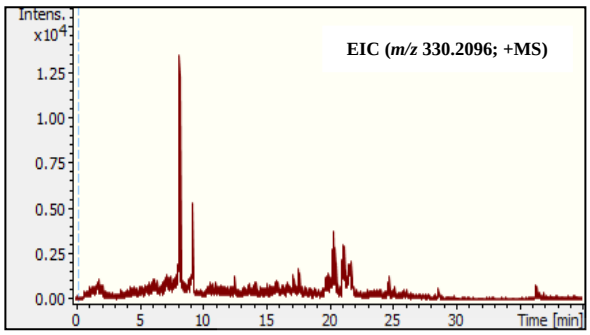
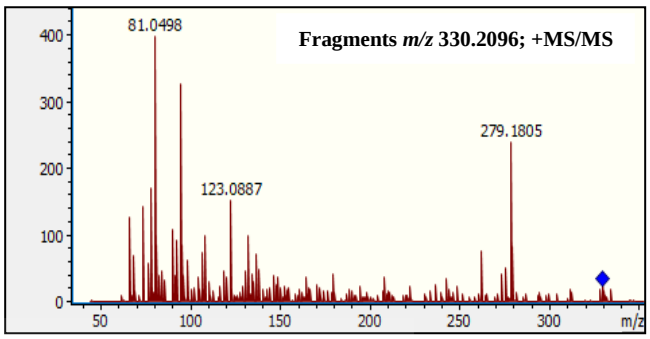
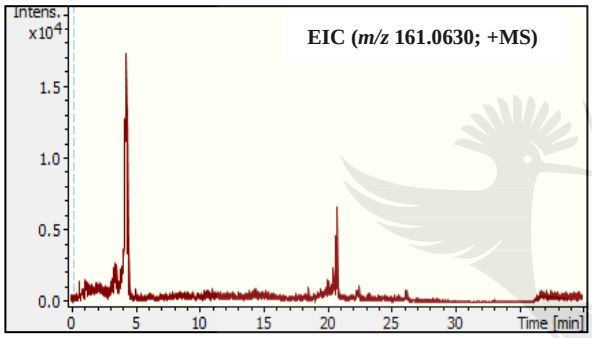
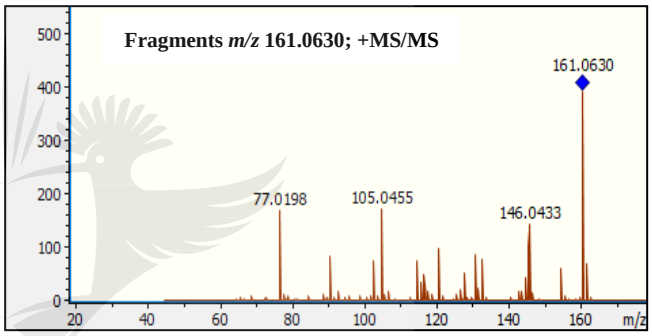
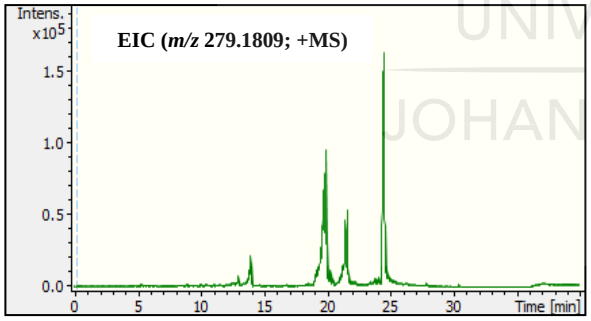
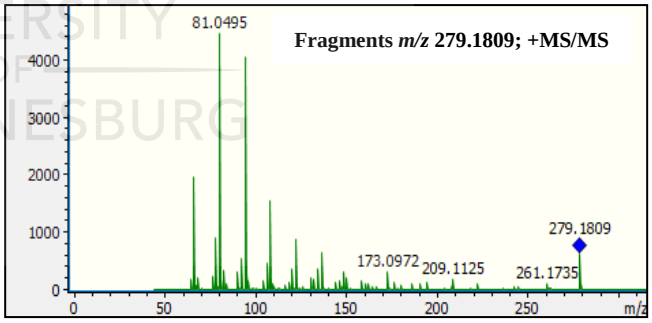
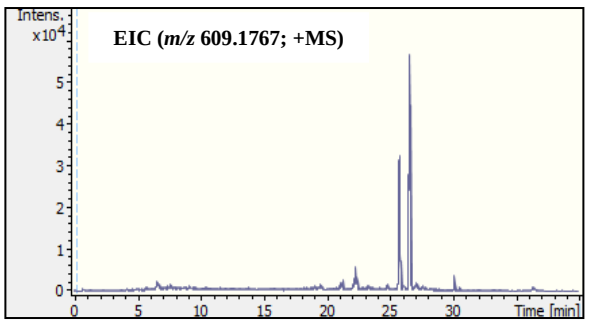
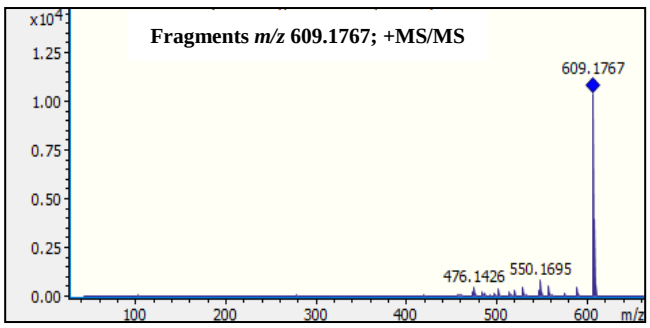
Table A12: Extracted ion chromatogram (EIC) of secondary metabolites from *Solanum mauritianum* stems – S5 Stem (left) and their MS^2 fragments (right).

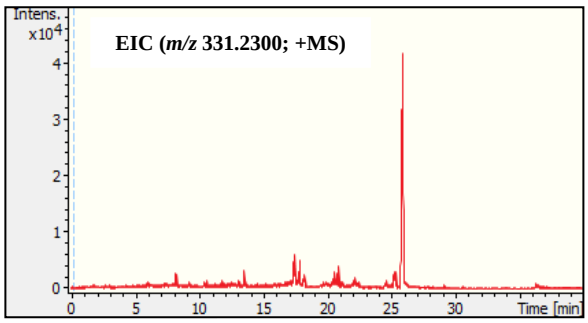
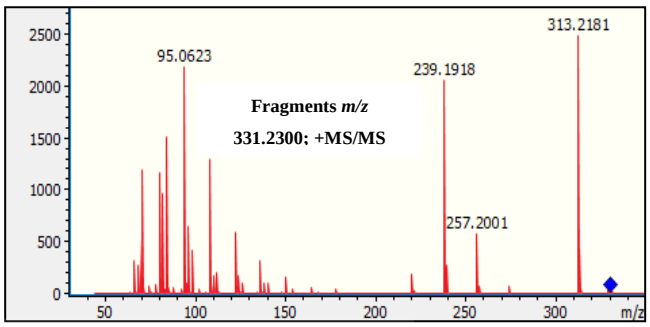
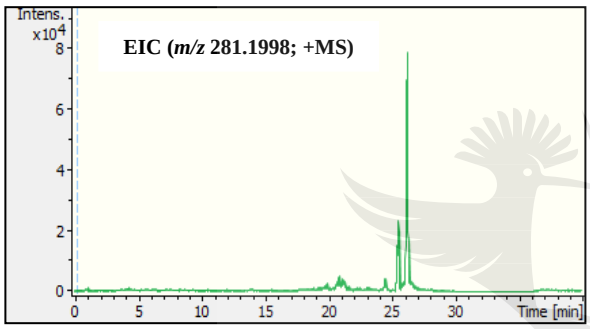
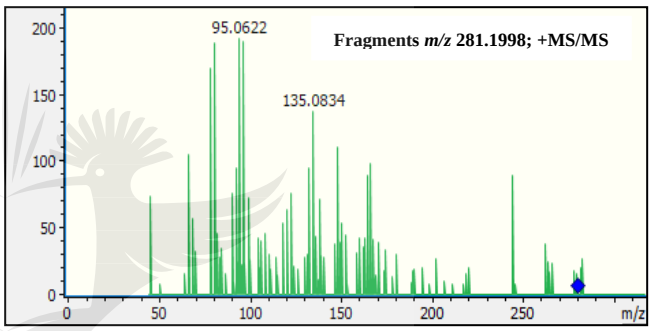
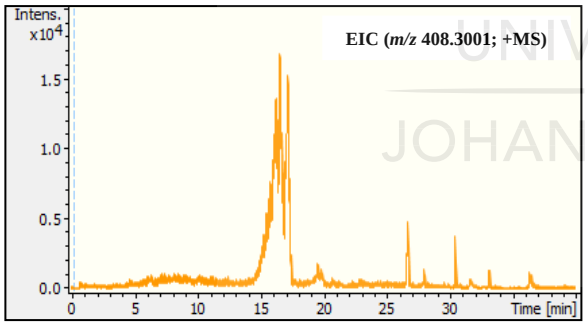
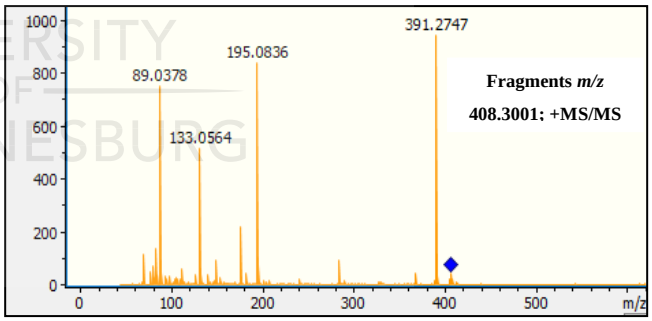
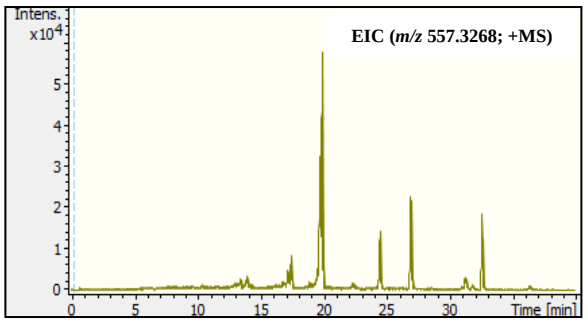
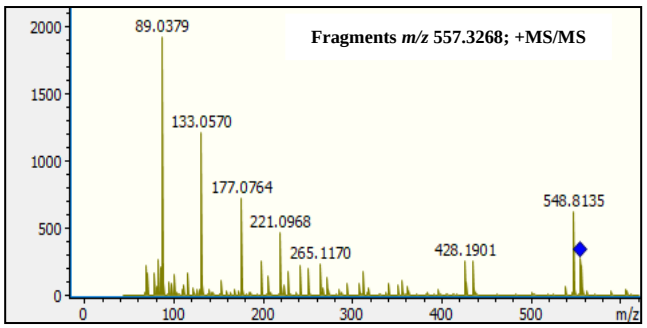
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Droserone	
	
Swainsonine	
	
Vicine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Cimifugin	
	
Gynocardin	
	
Solasonine	
	
α-Solanine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Imperialine	
	
α-Ergocryptine	
	
Solasodine	
	
Quercetin 3-O-sophoroside	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Montanol	
	
Licarin A	
	
Coniferyl alcohol	
	
Hypercalin B	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Hetisine	
	
Anatabine	
	
Lycocernuine	
	
Diosmin	
	

EIC: y axis = Intensity; x axis = Time (min)	MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)
Cannabielsoin	
	
Ibogamine	
	
Cassaidine	
	
Cucurbitacin E	
	

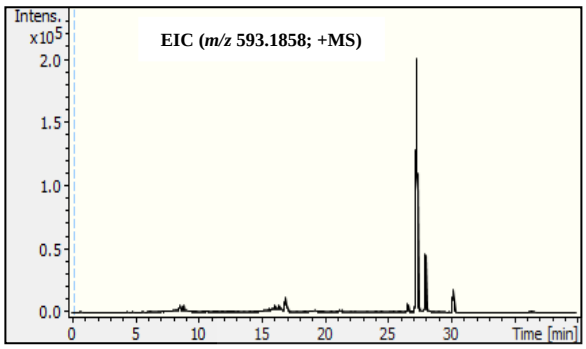
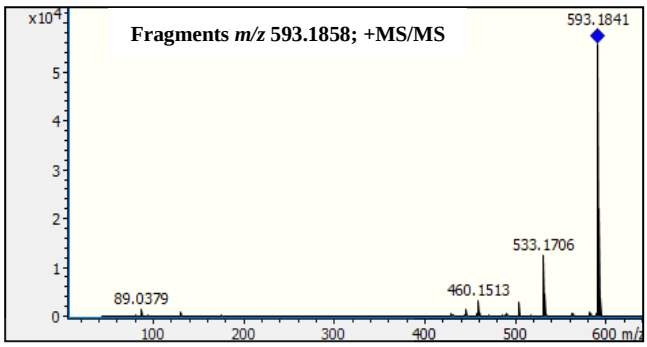
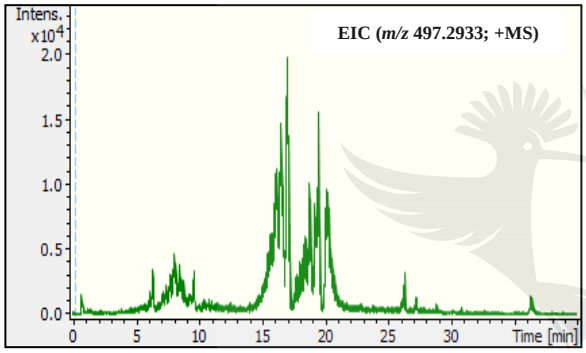
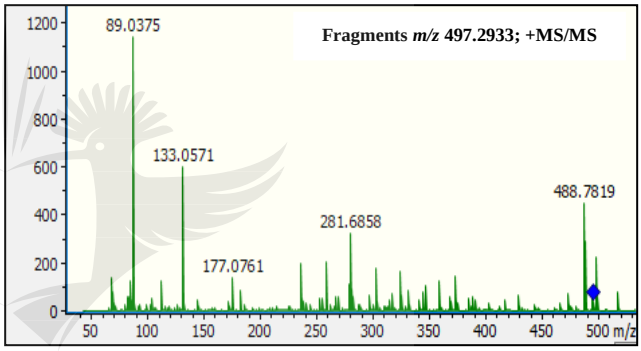
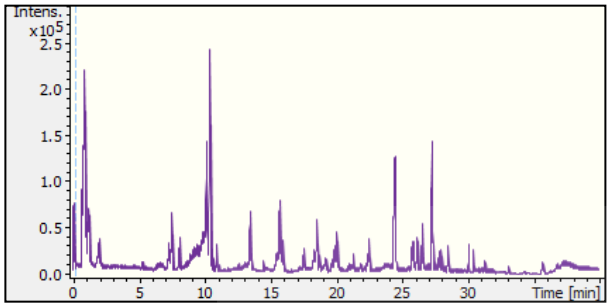
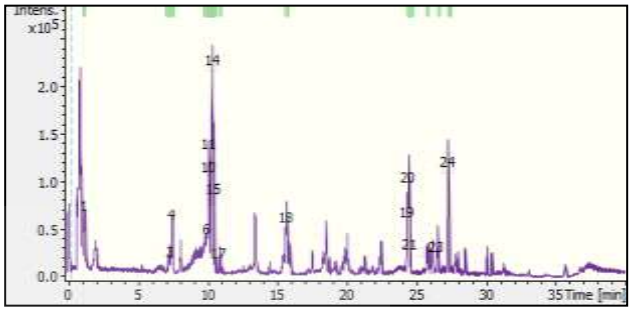
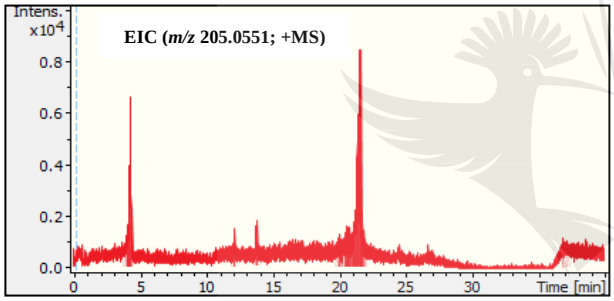
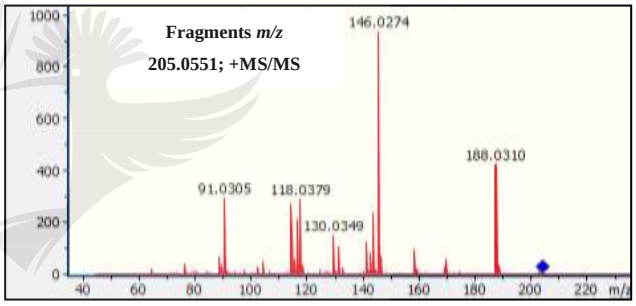
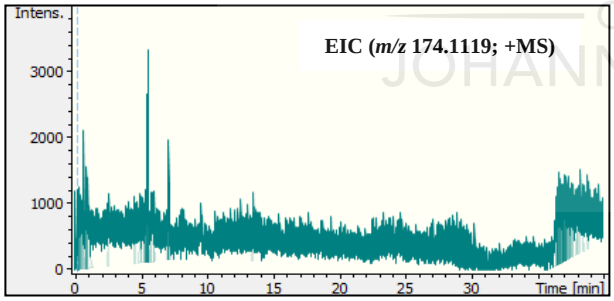
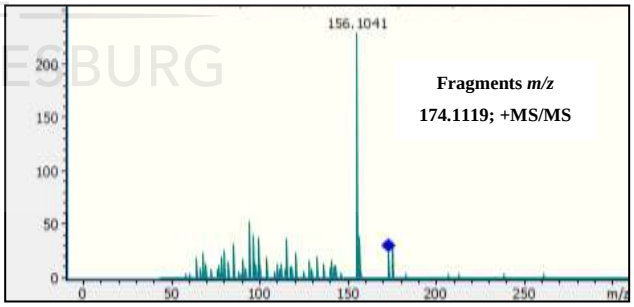
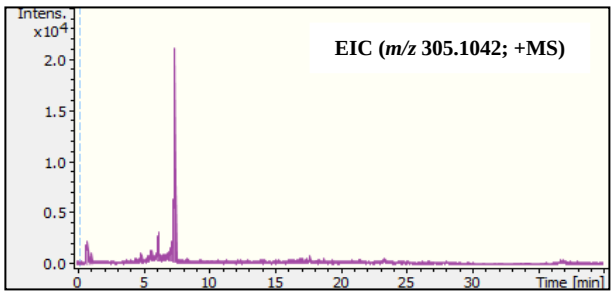
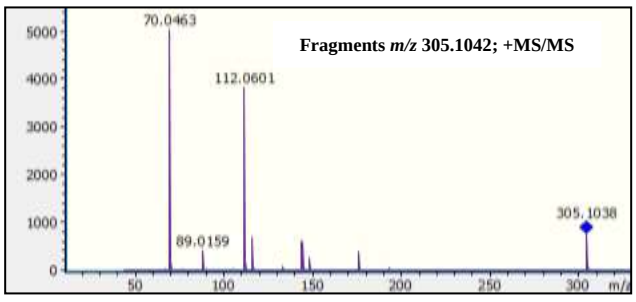
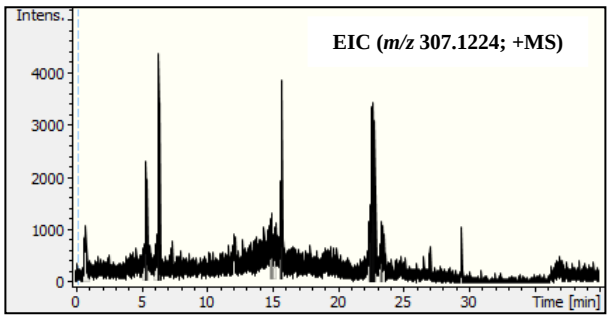
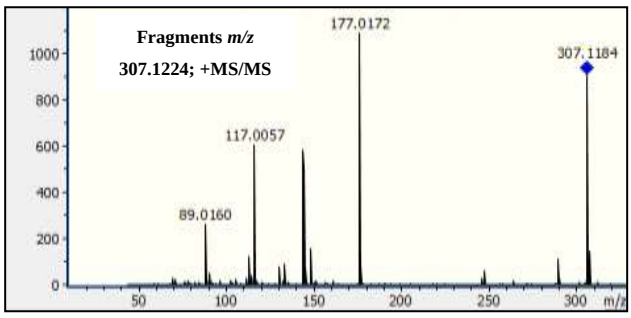
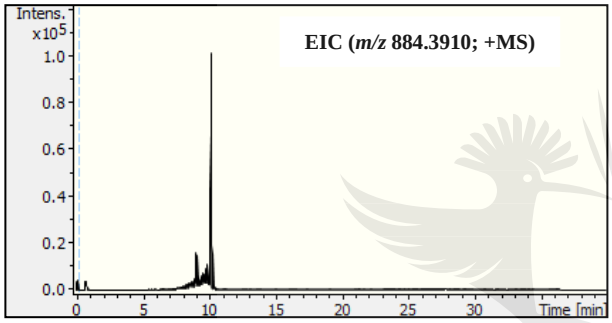
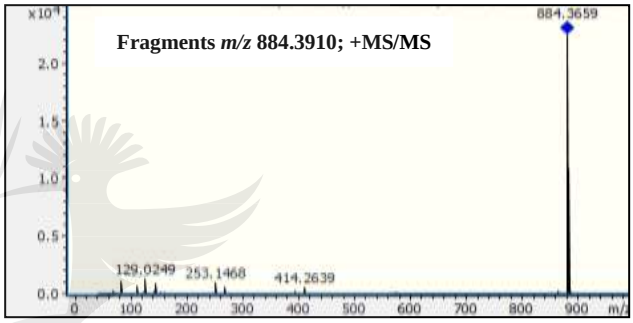
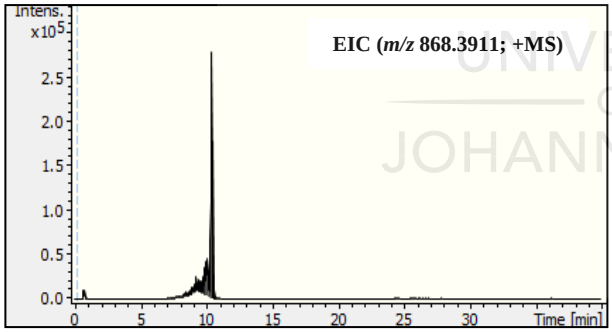
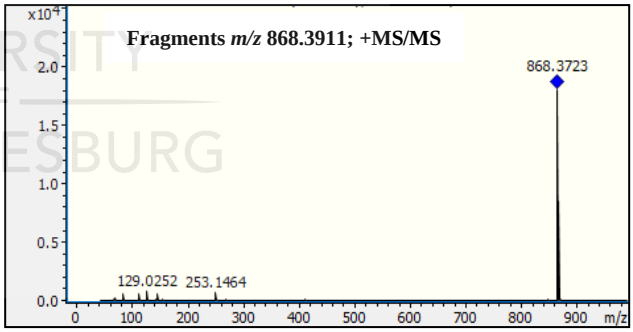
EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Isoswertisin 2"-rhamnoside	
	
Absinthin	
	

Table A13: Extracted ion chromatogram (EIC) of secondary metabolites from *Solanum mauritianum* leaves – S7 Leaves (left) and their MS^2 fragments (right).

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Base Peak Chromatogram (BPC)	
	
Droserone	
	
Swainsonine	
	
Vicine	
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Cimifugin	
	
Solasonine	
	
α-Solanine	
	
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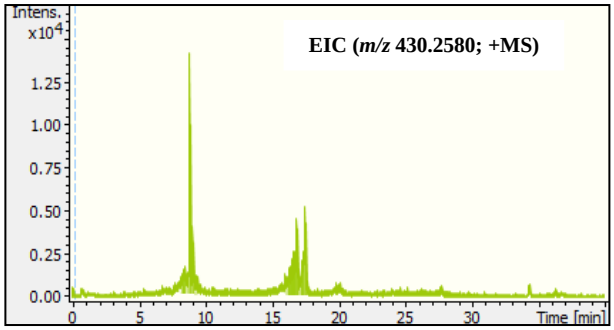	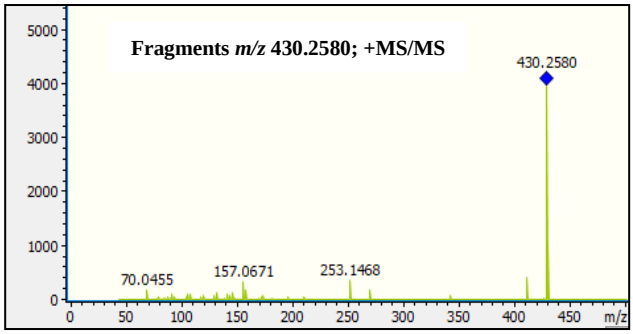

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
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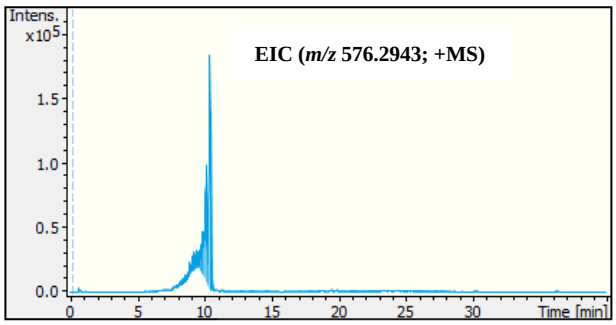	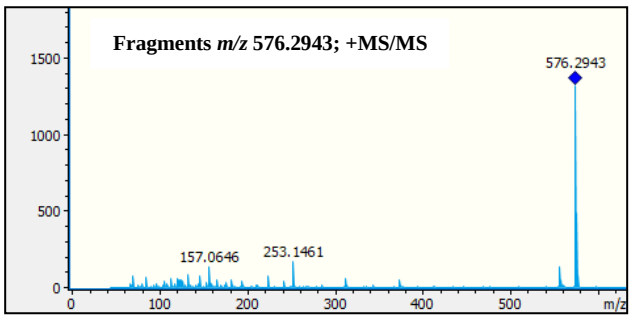
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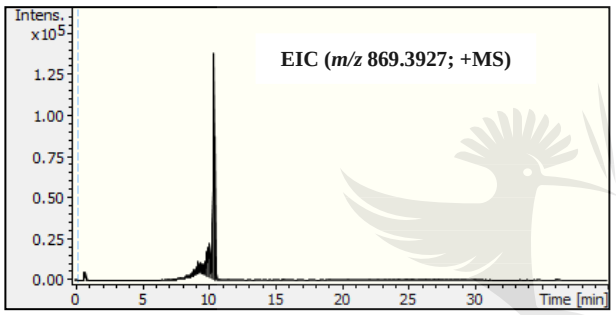	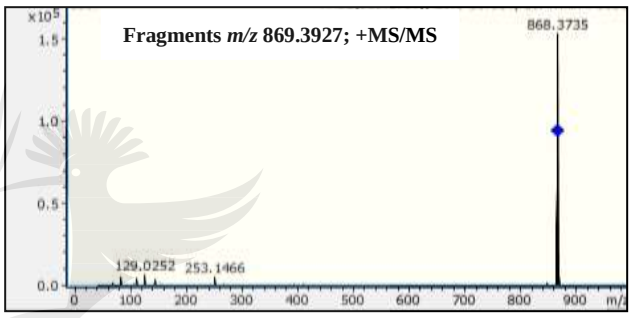
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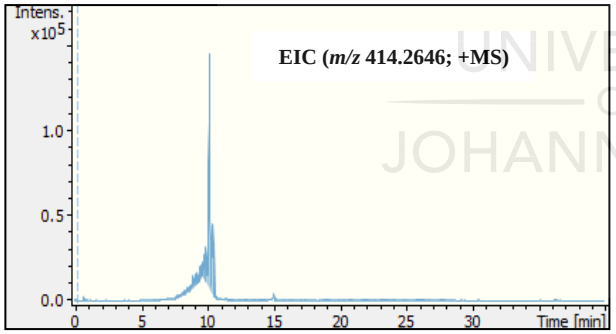	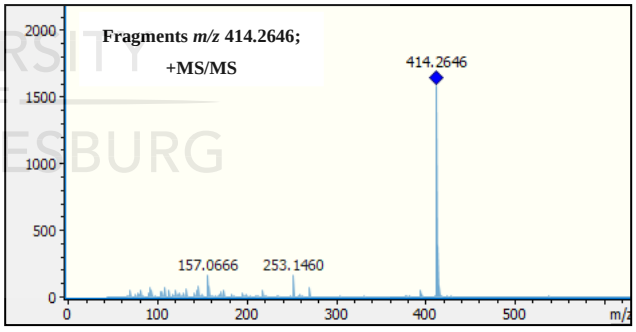
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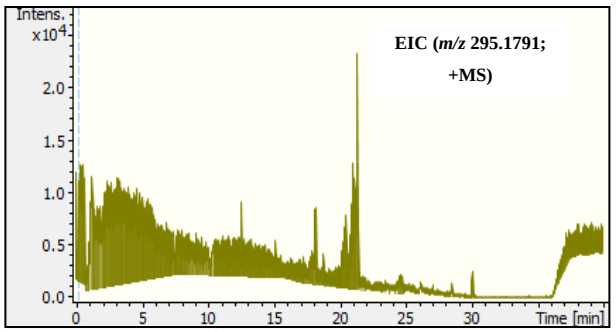
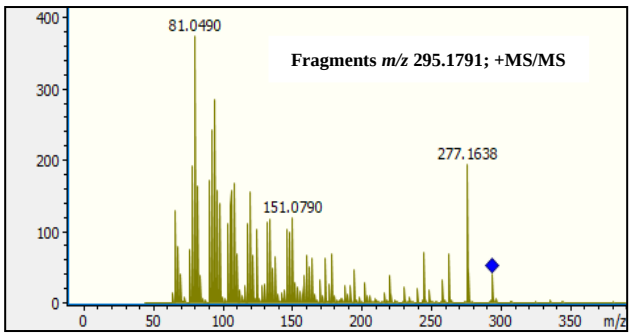
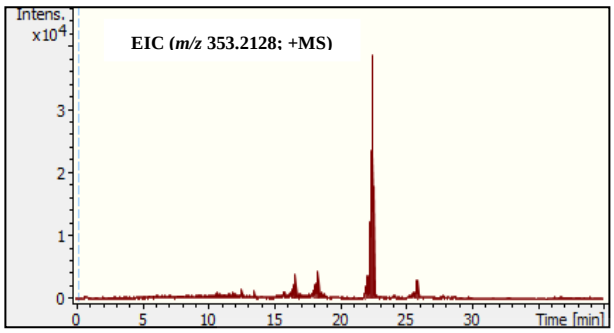
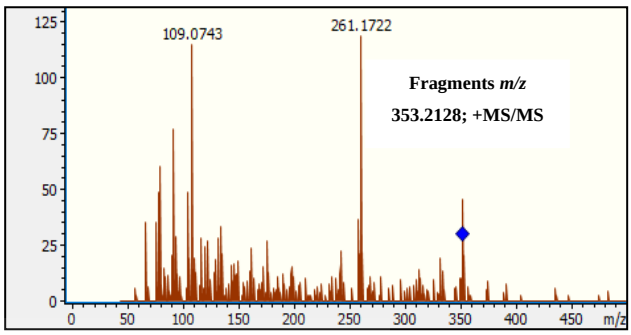
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Montanol	
	
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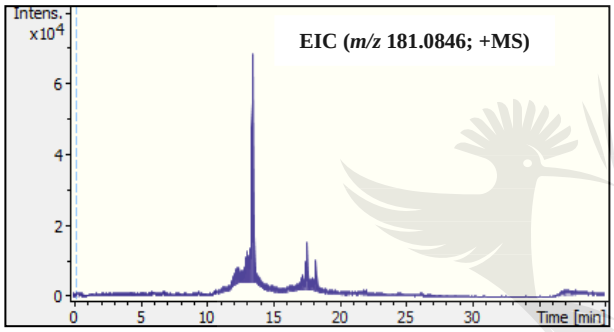	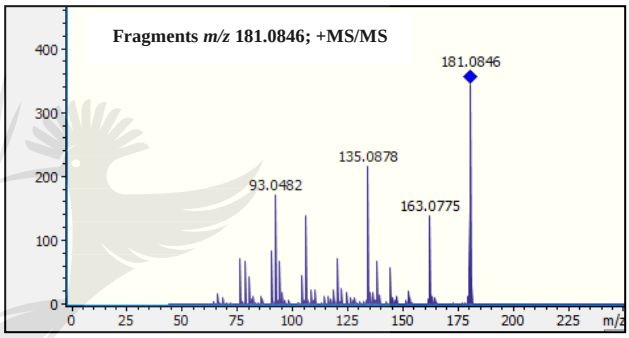
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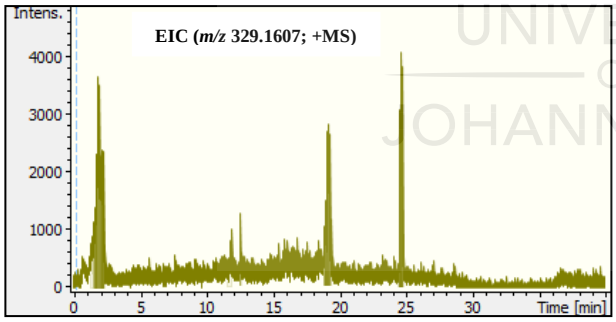	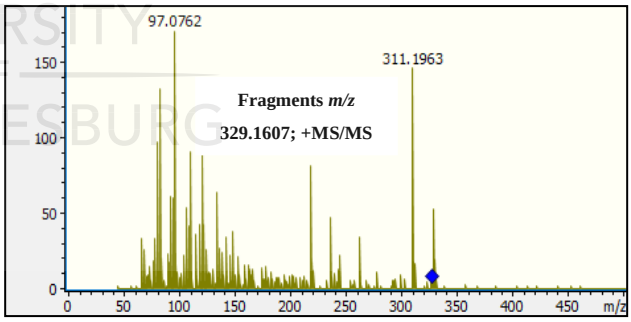
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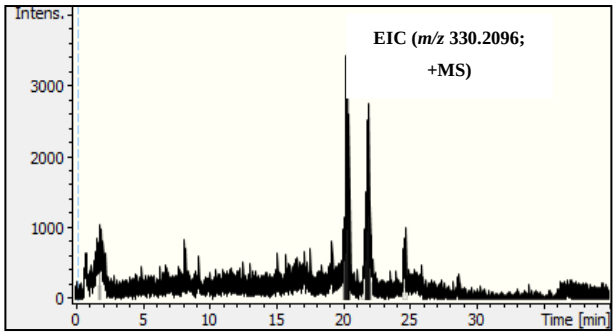
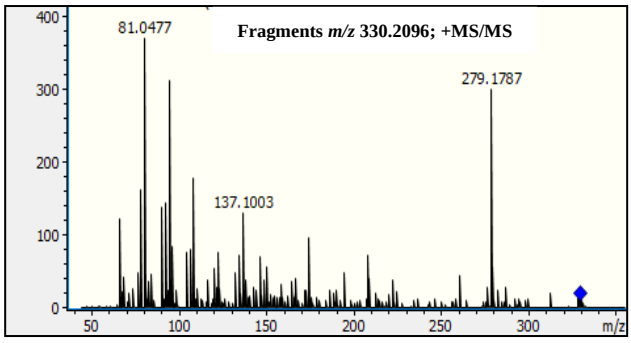
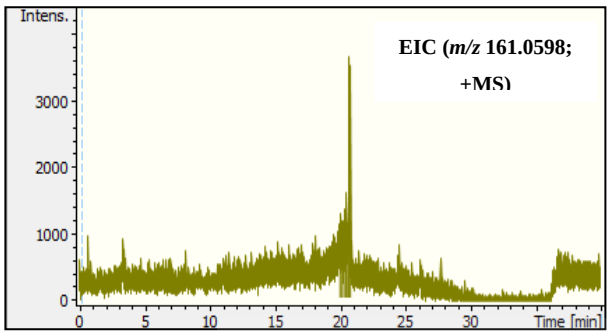
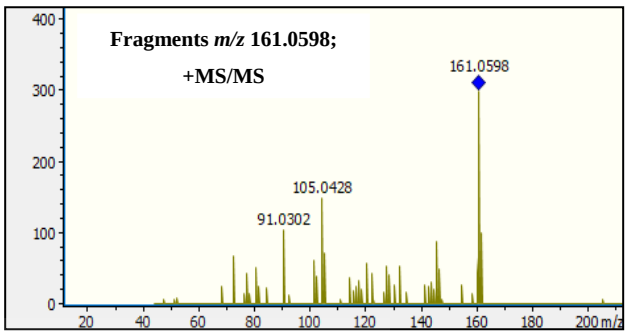
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Anatabine	
	
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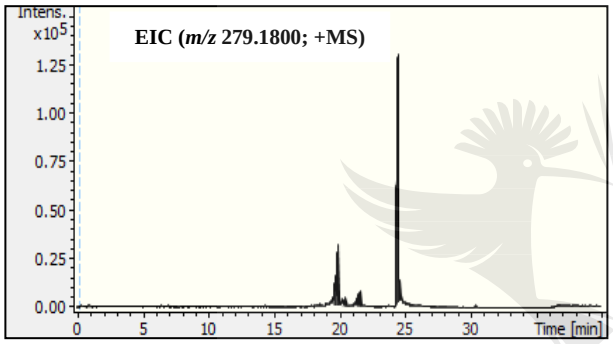	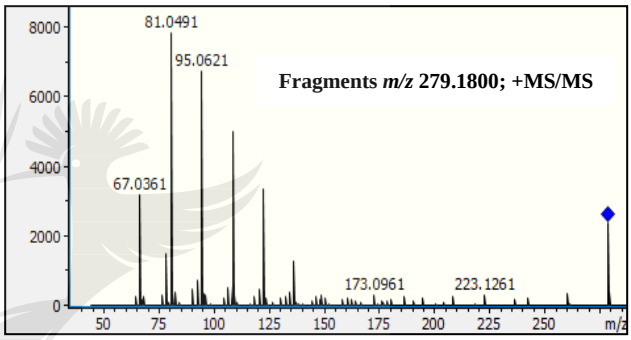
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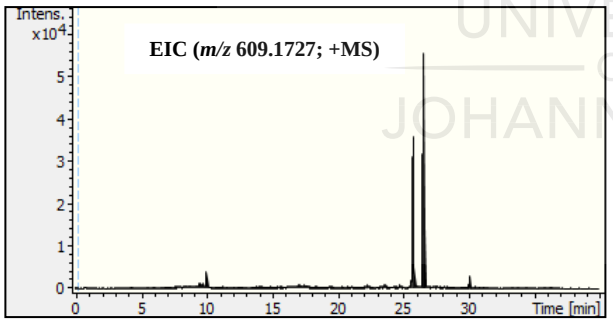	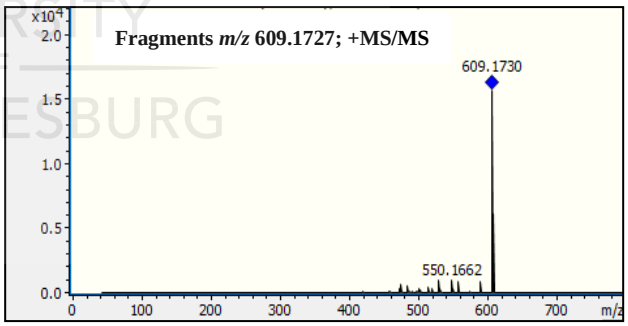
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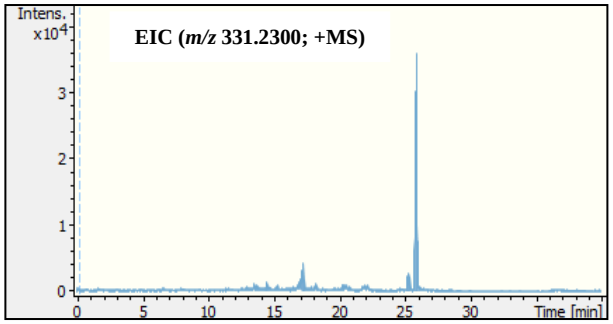
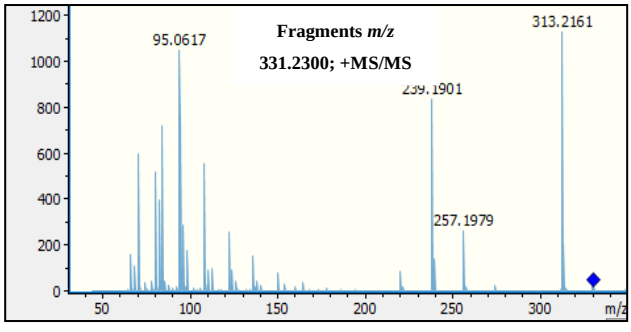
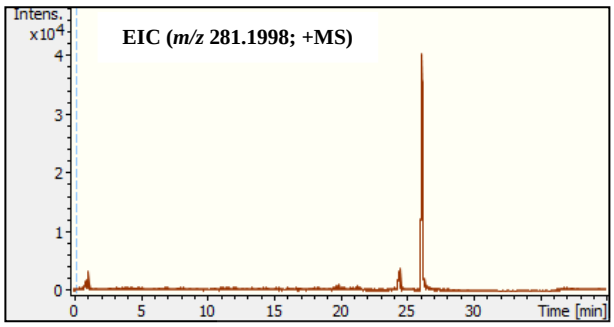
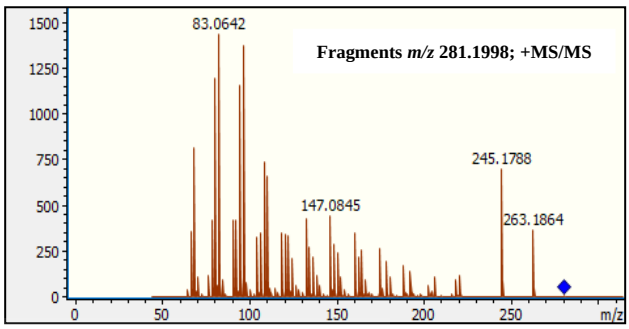
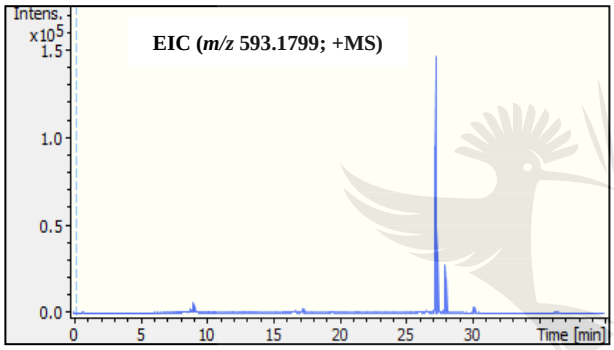
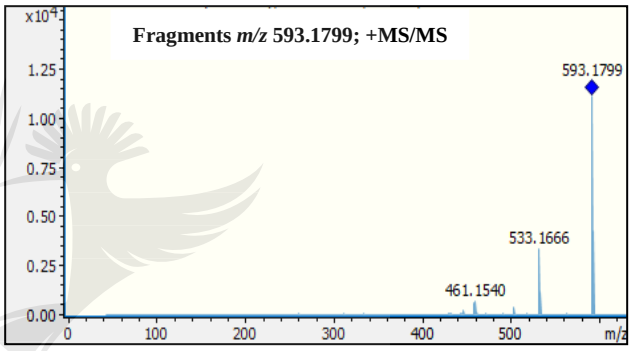
	

EIC: y axis = Intensity; x axis = Time (min)	**MS^2: y axis = Intensity; x axis = *m/z* (Mass to charge ratio)**
Ibogamine 	
Isoswertisin 2"-rhamnoside 	
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